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## **PHYSICOCHEMICAL MECHANISMS FOR THE REMOVAL OF INSECT WAX BY MEANS OF FINELY DIVIDED POWDERS<sup>1</sup>**

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### **INTRODUCTION**

ABOUT 95 per cent of the insect cuticle consists of a relatively thick inner part called the procuticle, composed of chitin, protein, and other compounds, and containing considerable quantities of water. Above the procuticle is the nonchitinous epicuticle, approximately  $1\ \mu$  in thickness, which consists of an inner hydrophylic protein zone, called the cuticulin, a middle zone composed of lipid (usually a solid wax), and an outer zone of shellac-like material, the so-called "tectocuticle" or "cement" (Richards, 1951).<sup>3</sup> Beament (1959) found that with some insects, wax in a volatile solvent migrates through the cement to form an outer layer of wax. It is the lipid of the epicuticle that protects insects from abnormally rapid loss of water.

A number of investigators have shown that when insects or mites crawl over films or layers of finely divided particles, particularly when the latter are deposited as dry dusts, they are susceptible to desiccation. This desiccation results from the removal by these particles of some of the very thin lipid layer of the epicuticle (Zacher and Künicke, 1931; Hockenyos, 1933; Germar, 1936; Zacher, 1937*a*, 1937*b*; Chiu, 1939; Alexander *et al.*, 1944; Kalmus, 1944; Parkin, 1944; Wigglesworth, 1944, 1945, 1947*a*, 1947*b*, 1957, 1958; Beament, 1945, 1958; Hunt, 1947; Cotton and Frankenfeld, 1949; David and Gardiner, 1950; Bartlett, 1951; Helvey, 1952; Glynne Jones, 1955; Holdgate and Seal, 1956; Nair, 1957; Ebeling and Wagner, 1959; Wagner and Ebeling, 1959; Tarshis, 1959, 1960, 1961; and Micks, 1960).

Some investigators have attributed the desiccating action of finely divided powders primarily or entirely to their ability to remove the protective lipid layer by abrasion (Wigglesworth, 1945, 1947*a*, 1958; Beament, 1945; Hunt, 1947; and David and Gardiner, 1950). Beament (1959) later reiterated his belief that stationary adsorptive powders applied to the "primary" wax layer do not increase its permeability to water, but that when applied

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<sup>3</sup> See "Literature Cited" for citations, referred to in the text by author and date.

to the wax above the "cement," in a wax-cement-wax system, they increase water loss to a rate that is higher than that of the same cuticle with only a primary wax layer. Alexander *et al.* (1944) found that abrasive powers were more effective against the adults of some species of beetles and sorptive powders were more effective against the adults of others, as well as the larvae of all species tested. Other investigators, particularly in later years, have recognized that adsorption can be the dominant factor in the removal of lipid from most insect species, provided the powders used possess the required physical characteristics for a high degree of sorptiveness for wax. The majority of these investigators (Hurst, 1948; Helvey, 1952; Glynne Jones, 1955; Nair, 1957; and Ebeling and Wagner, 1959) demonstrated that the ability of finely divided powders to remove the epicuticular wax of the insects they investigated increased with increasing sorptiveness. All the highly effective powders have been nonabrasive.

There has been considerable controversy as to whether powders can cause lethal dehydration of insects entirely, or even in part, by adsorption of the solid wax. The adsorption of the "mobile grease" of the cockroach has been conceded (Wigglesworth, 1945; Beament, 1945). This controversy is of more than academic interest. Obviously, if the protective wax can be removed only by abrasion, the use of inert powders or dusts for the control of insects through desiccation must be confined to those species whose habitats and habits make them susceptible to the action of abrasives, primarily the granary weevils. In fact, powders whose action is entirely abrasive have been used successfully, in limited commercial practice, only against granary weevils, which abrade their bodies as they crawl among the kernels of grain. Even for such insects, however, the nonabrasive, but highly sorptive, silica aerogels have proved to be superior (Cotton and Frankenfeld, 1949). On the other hand, if the epicuticular wax can be removed by adsorption, many species, with a wide variety of habitats and habits, will be susceptible to the lethal effect of the sorptive powders. The insect need only accumulate the powder on the underside of its body as it crawls over the dusted surface (Ebeling and Wagner, 1959).

If insects are immobilized by death or otherwise before a sorptive powder is applied, the initiation of water loss is delayed for several hours, but then proceeds at a rate far in excess of that of undusted insects (Ebeling and Wagner, 1959).

It was the primary purpose of this investigation to demonstrate the adsorption of insect wax by means of finely divided powders, visually and gravimetrically, and to propose a physicochemical mechanism by which this action takes place. For ease of manipulation and for purposes of standardization, the experiments were made primarily with beeswax as a representative insect lipid. However, the removal of dyed lipid from the insect epicuticle by means of sorptive powders was photographically recorded.

An additional aim in this investigation was to correlate certain physical characteristics of finely divided powders with their ability to adsorb insect wax and with their insecticidal effectiveness.



## MATERIALS

One of the premises in this investigation was that information obtained regarding the adsorption of beeswax would be applicable in a general way to the wax layers of many insect species. The chemistry of beeswax has been investigated in great detail, particularly by Warth (1956). It has the general composition of all insect waxes investigated to date, primarily comprising esters, wax acids, and hydrocarbons. However, its free alcohol content is very low (1 to 1.25 per cent), in contrast to the high quantities claimed for various insect waxes by previous investigators. Warth believes that although alcohols are initially produced in large quantity, they are soon oxidized by free oxygen to the corresponding acid molecules.

In one analysis of beeswax, esters comprised 71 per cent of the total and had a melting point of  $64.5^{\circ}$  C. Among the simple esters, the most abundant was myricyl palmitate,  $C_{15}H_{31}CO.O.C_{26}H_{53}$ . Free wax acids, mainly saturated, made up 13.5 to 14.5 per cent of the total wax. Hydrocarbons, mainly the saturated hentriacontane,  $C_{31}H_{64}$ , with a melting point of  $68.4^{\circ}$  to  $69.0^{\circ}$  C., constituted 10.5 to 13.5 per cent (Warth, 1956).

Since the glands that secrete the beeswax of commerce are merely specialized epidermal glands homologous with those that secrete the wax covering the epicuticle of the bee, it is reasonable to presume that their product is practically the same. Glynne Jones (1955) found that the melting point of the wax in the epicuticle of the bee is practically the same as that of extracted beeswax ( $62^{\circ}$ – $65^{\circ}$  C.).

The specific gravity of beeswax is 0.95 to 0.96. When distilling off 2.1 per cent of a beeswax sample in a ball-tube distillation apparatus at  $210^{\circ}$  to  $230^{\circ}$  C. and 0.08 mm. Hg, the writer found that, upon cooling, the material solidified, indicating no liquid fractions.

For the purposes of the present investigation, it was considered desirable to work with films of beeswax on glass or plastic vials. Films of different thicknesses, ranging from 0.32 to  $65\ \mu$ , were obtained on the outer surfaces of stoppered vials by dipping them into solutions of beeswax in carbon tetrachloride in concentrations ranging from 1.5 to 50 per cent, or by dipping them into melted beeswax, usually the yellow beeswax of commerce. However, some work was done with a white wax, filtered and refined without the use of acid and with a minimum of heat. This wax is free of pollen, propolis, and other contaminants found in the yellow beeswax of commerce.

In one experiment, U.S.P. household paraffin was compared with beeswax in regard to loss of weight of the wax films by adsorption and by exposure to high temperatures. Paraffin is a mixture of solid hydrocarbons obtained from petroleum and has the general formula  $C_nH_{2n+2}$ , with the number of carbon atoms ranging from 22 to 30. The Parowax used in the present experiments has a melting point averaging about  $51.5^{\circ}$  C. and contains fractions ranging in melting point from  $37^{\circ}$  to  $64^{\circ}$  C.

The finely divided powders used in the experiments are described below. Some of the more pertinent physical characteristics are also listed in table 1.

(1) **Silica aerogel SG-68.** Silica gels are well-known dehydrating agents and adsorbents. They are amorphous, nonabrasive powders, formed as a



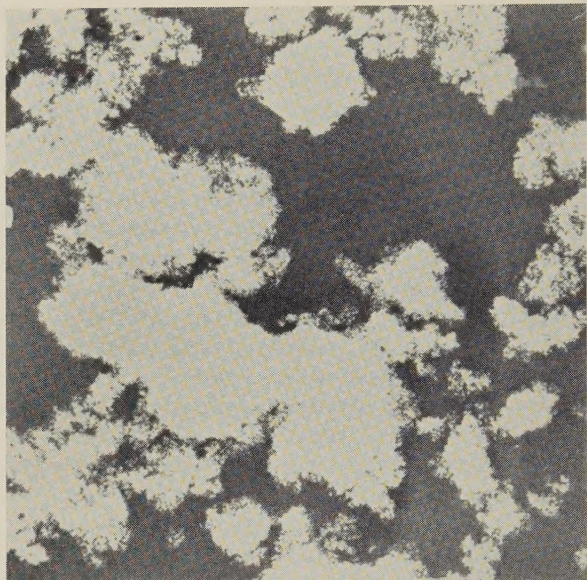


Fig. 1. Electron micrographs of silica aerogel powder SG-67, showing lacy structure evident at edges of the agglomerates, which comprise many minute particles approximately  $0.01$  to  $0.05\ \mu$  in diameter.  $\times 12,600$ .

result of the primary chemical reaction of sodium silicate and sulfuric acid. A firm hydrogel is formed which is washed free of reaction salts. A granular, dehydrated silica gel results that can be reduced to very small particle size. Because of their noncrystalline structure, high purity (99.5 per cent  $\text{SiO}_2$ ), and chemical inertness, silica gels are not injurious to human beings. They do not cause silicosis, but respirators are recommended for those who work for extended periods with the powder, to prevent irritation resulting when an appreciable amount of the material is inhaled.

The silica gels of lowest bulk density, and consequently of greatest porosity, are called aerogels. SG-68 is an extremely light, fluffy silica aerogel, with 82 per cent of the aggregates ranging from  $0.1$  to  $3.2\ \mu$  in diameter, but electron micrographs show that these comprise myriads of tiny ultimate particles only  $0.01$  to  $0.05\ \mu$  in diameter (fig. 1, showing SG-67). SG-68 has an average pore diameter of  $115\ \text{\AA}$  and a surface area (specific surface) of 300 square meters per gram, and will adsorb 300 per cent of its weight of linseed oil. It has a pH of 7.0. The pH of silica gels and aerogels is based on a 5 per cent slurry in distilled water.

(2) **Silica aerogel SG-67.** This product is the same as SG-68, except for 4.7 per cent of ammonium fluosilicate present in less than a continuous monolayer. It has a density of 4.5 pounds per cubic foot, as packed, and a pH of 2.6.

The fluoride is added to the silica hydrogel in the process of manufacture and was originally added to decrease the caking characteristics of the gel and to produce an improved silica flattening agent. However, the fluoride greatly improves the silica aerogel as an insecticide by causing the particles



to have a positive charge, thus enhancing their depositing ability and adherence when applied as a dust. In addition, silica aerogel appears to have the ability to disrupt the outermost layer of the epicuticle, which Richards (1957) has found to be the major cuticular barrier to ions. The penetration of the ion barrier, plus the adsorption of lipid, appears to allow the inorganic water-soluble toxicant to contact the water-bearing portion of the cuticle and to act as a contact insecticide (Ebeling and Wagner, 1959).

(3) **Silica gel AL-1.** This material differs from the two silica aerogels described above in having about three times higher density, a greater particle size, a much smaller pore size, and greater specific surface. It has a greater ability to adsorb water. Its pH in water slurry is 2.8.

(4) **Silikil.** This is a silica gel with a relatively low degree of wax adsorbency, low specific surface (36 meters<sup>2</sup>/g.), relatively high bulk density (approximately 3.5 times as high as SG-67), and with 85 per cent of the particles ranging from 1 to 5  $\mu$ . It has a pH of 4.25 in 5 per cent aqueous slurry.

(5) **Activated carbon.** This black powder is made from lignite. Ninety per cent passes through a 325-mesh screen. It has the largest pores of all powders used in this investigation and also a large specific surface (545 meters<sup>2</sup>/g.). The pH of the water extract is 9 to 11.

(6) **Olancha Clay.** One of four fuller's-earth-type clays, out of 162 finely divided powders tested, that were found to have outstanding ability to kill drywood termites by desiccation (Wagner and Ebeling, 1959). It is a montmorillonite clay with 58.6 per cent SiO<sub>2</sub> and 12.8 per cent Al<sub>2</sub>O<sub>3</sub>. It has a Scott Volumeter density of 16 to 18 pounds per cubic foot, with 91 per cent passing through a 325-mesh screen, a linseed-oil adsorption of 120 per cent, and a pH (in 1-5 slurry) of 6.75.

(7) **Attaclay.** This is another fuller's-earth-type clay with considerable ability to kill insects by desiccation. It is an attapulgitic clay with 67.0 per cent SiO<sub>2</sub>, 12.5 per cent Al<sub>2</sub>O<sub>3</sub>, and 11 per cent MgO. It has a Scott Volumeter density of 17.8 pounds per cubic foot, 85 per cent passing through a 325-mesh screen, a linseed-oil adsorption of 119 per cent, and a pH (in water slurry) of 6.9 to 7.1.

(8) **Insecticide Grade Pyrophyllite.** This material contains 75.0 per cent SiO<sub>2</sub> and 19.5 per cent Al<sub>2</sub>O<sub>3</sub>; a bulk density (fluffed) of 30 pounds per cubic foot; and 93.5 per cent passes through a 325-mesh screen. Pyrophyllite particles have no internal porosity or surface, and this powder can adsorb only 50 per cent of its weight of linseed oil. Its pH in water slurry is 6 to 7.

(9) **Barytes.** This material is used as a filler for rubber. It is 86.18 per cent BaSO<sub>4</sub>. The powder is heavy, white, and 99.7 per cent passes through a 325-mesh screen.

(10) **Molecular Sieves.** These synthetic zeolites are alkali metal aluminum silicates, quite similar to many natural clays and feldspars. With most hydrated materials of this type, when the water of hydration is driven off, the crystal collapses or becomes rearranged. With Molecular Sieves, however, the structure of the crystal remains unchanged, which results in a network of empty pores and cavities that comprise about half the total volume of the crystals. Molecular Sieves have a pH in water slurry of approximately 10.

(11) **Sand.** Approximately equal parts of quartz and feldspar. The sand used was angular in shape and was ground to pass through a 200-mesh screen.

(12) **Carborundum.** Carborundum (silicon carbide—SiC) is second only to diamond dust in abrasiveness. Because of the limitations in the manufacture of screens, all numbers below 240 "grit" are nominal rather than absolute designations of mesh size. The particle sizes, for the bulk of the powder, are given for grits 400 and 600 in table 1.

### Observation of Adsorption from Beeswax Layers with the Aid of a Microscope

According to Azaroff and Buerger (1958), most waxes are crystalline. In the present investigation, it was shown by X-ray crystallography, according to the powder method, that beeswax contains both crystalline and amorphous material. Although beeswax is a solid substance, the same physicochemical laws that pertain to the adsorption of ordinary liquids would be involved in the adsorption of the amorphous constituents. (Whenever adsorption of wax is referred to in the present paper, amorphous wax is implied.)

Most of the physical and organic chemists who were consulted regarding the apparent removal of wax from the epicuticle of insects by adsorption, as indicated by staining techniques (Ebeling and Wagner, 1959), were skeptical of the validity of this conclusion because of the relatively rapid rate of removal of the wax reported in the experiments in question. The numbers of minutes for 50 per cent kill of insects by placing them on a film of one of the silica gels, for example, were as follows: ants, 10; *Drosophila melanogaster*, 19; German cockroaches, 92; drywood termites, 152; and houseflies, 185 (Ebeling and Wagner, 1959). Therefore, it was considered desirable to obtain visual evidence of the rate of adsorption of insect wax and its migration over solid surfaces.

The ability of certain sorptive powders to adsorb solid wax was easily verified by direct observation. A silica aerogel (SG-67) was allowed to settle out from a cloud of dust formed in a 1,000-ml. beaker onto two microscope slides, one covered with a twenty-four-hour-old film of white beeswax about 65  $\mu$  in depth. When examined with a stereoscopic microscope at  $\times 96$  magnification, the aggregates of silica aerogel on these slides were at first a translucent gray when resting on glass or wax. They retained this appearance when resting on glass. On beeswax, the smaller aggregates of the powder became white within an hour at 22° C., indicating wax adsorption. Within two hours, the wax had diffused over the entire surface of some aggregates, rising 10 to 20  $\mu$  up into the wax layer on which they were resting. Over a period of a few days, nearly all silica aerogel aggregates had gradually turned white, beginning at their bases and extending upward as far as 30 to 40  $\mu$ . Figure 2 contains photomicrographs of layers of silica aerogel on glass and on white beeswax three days after it was applied. The color of the waxed silica was similar to that of the freshly broken surface of a cake of beeswax. It contrasted so sharply with the color of the unimpregnated silica that the exact point to which the wax had migrated was dis-



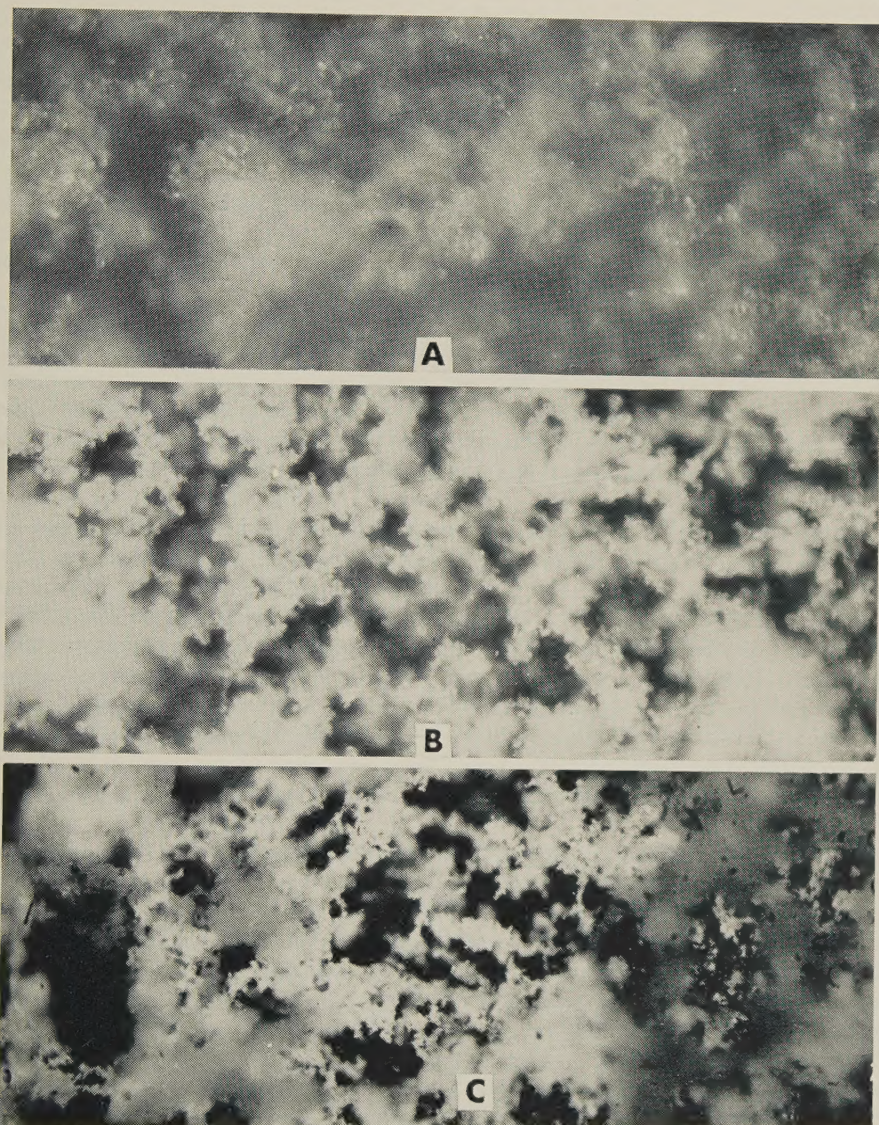


Fig. 2. Adsorption of insect wax by silica aerogel SG-67. *A*, Silica powder resting on glass, showing, out of focus, the gray, fluffy masses and, in focus, the gray, translucent, granular formations. *B*, Silica powder after twenty-four hours on beeswax, showing, out of focus, the typical, wax-impregnated, white, fluffy masses and, in focus, the white, opaque, granular formations, also typical of wax-impregnated silica. *C*, Silica powder after twenty-four hours on abdominal tergite of an American cockroach, showing, in focus, the bottom layer of lipid-impregnated, white, opaque, granular formation, and above this and out of focus, the gray masses of unimpregnated silica not reached by the available cockroach lipid.

In *A* and *B*, the focus is slightly above the substrate, and the out-of-focus masses of silica are farther from the substrate (nearer the camera); in *C*, the focus is on the layer of silica aggregates immediately adjoining the substrate.



tinely defined. The portion of the silica that is completely in focus has a translucent, gray, granular appearance and a lacy design. This changes to an opaque white after it has been covered with a film of beeswax. This change in appearance can be readily seen in the figure.

After the silica aerogel resting on wax had turned white, it could be washed off with water, filtered, and the wax could be extracted with hot chloroform. Evaporation of the solvent on a glass slide left a wax deposit on which water drops had a 90° contact angle. Similar treatment of freshly dusted waxed slides did not result in a perceptible wax residue on slides on which the solvent was evaporated.

In order to facilitate observations, white beeswax was melted and dyed by the addition of 2.5 per cent by weight of Oil-Soluble Red. Microscope slides were dipped into the melted wax, in order to form a film on the glass, and then they were set aside for twenty-four hours. Powders were then deposited on the dyed wax films as before, by allowing them to settle out from clouds of dust formed in 1,000-ml. beakers placed upside down over the slides.

At 23° C., many of the smallest aggregates of the silica aerogels SG-67 and SG-68 were already completely saturated with the wax in fifteen minutes, as indicated by their red color. Subsequent diffusion of the wax over the silica aerogel particles proceeded approximately as described above with reference to the undyed wax. The wax did not saturate as large aggregates of Olancha Clay or Attaclay as of the silica aerogels. The powders that are least effective in killing insects by desiccation, such as pyrophyllite, tale, sulfur, and walnut-shell flour, did not adsorb wax, at least not to the extent of becoming visibly colored.

None of the powders changed color when placed on a layer of the powdered dye alone.

At a temperature of 23° C., silica aerogel adsorbed wax more rapidly at 15 and 42 per cent relative humidity than at 70 per cent. However, at 70 per cent relative humidity, all the smaller aggregates were saturated with wax in two hours. At 100 per cent relative humidity, the silica particles became visibly wet. Nevertheless, within a two-day period, many of the aggregates took on various shades of pink, ranging to red on some of the smaller aggregates. All the aggregates eventually became red. This discoloration cannot be attributed to the independent effect of the dye, for this powder is not soluble in water.

Dr. J. J. Bikerman has suggested to the writer that the apparent movement of the dyed wax over wet silica may be explained by the "Marangoni effect," which pertains to the movements in the surface of water caused by local variations in surface tension (Bikerman, 1958). In the present instance, the film of water on the silica particles might dissolve some wax molecules (presumably primarily the acids). The surface tension of the water would be reduced at the point of contamination. The tendency in such a circumstance is for the contaminating substance to be dispersed over the entire surface area of the water; in this case, the film covering the silica particles.

It is of interest to note that Ebeling and Wagner (1959) found that in seven hours, drywood termites lost 36.7 per cent of their body weight from



loss of water when placed on silica aerogel SG-67 at 100 per cent relative humidity, compared with 2 per cent loss when not in contact with the powder. This appears to be explainable only on the basis of the removal of cuticular lipids, even in a water-saturated atmosphere. It also indicates that, when the lipid protective covering on their epicuticle is removed, insects can lose water at an abnormal rate, even in a water-saturated atmosphere. Tarshis (1961) made similar observations with regard to cockroaches, fleas, and cheese mites.

### Observation of Adsorption from Insect Cuticles

If, instead of dipping glass slides into melted wax, they are dipped into a hot solution of 3 per cent beeswax in carbon tetrachloride, films can be produced of about the same thickness as the insect lipid layer,  $0.4\ \mu$  or less. Only the smallest particles of a sorptive dust layer on such a thin wax film will then become completely covered with wax. These can be most easily seen if the wax is dyed. The larger aggregates adsorb some wax at their interfaces with the substrate, but often the quantity is not sufficient to be visible from above. As might be expected, the minute quantity of wax that is present in the epicuticle of most insects is also sufficient to cover only the smallest sorptive particles.

The wax of the insect epicuticle can be dyed with acetone solutions of Oil-Soluble Red, but the acetone, when evaporating, tends to bring to the surface additional quantities of lipid from below. To avoid this, the wax layers can be dyed by allowing a fine powder of Oil-Soluble Red dye to settle, from a confined cloud, onto the bodies of the insects. The dye is dissolved by the wax and spreads from each particle or aggregate until in about twenty-four hours the wax layer is quite uniformly dyed. The excess dye powder can then be blown and washed off.

A layer of SG-67 was allowed to settle on the bodies of *Eleodes* beetles, termite nymphs, fleas, and cockroaches that had been exposed to dye powder at  $27^{\circ}\text{C}$ . for twenty-four hours. With the exception of the newly killed cockroaches, which had a liberal supply of readily adsorbed lipid, the amount of wax adsorbed by the powder was as small as that adsorbed from the thin beeswax films. Only the smallest dust particles were uniformly colored by the dyed insect lipid. The adsorption was recorded by means of color transparencies.

Cockroaches had such an abundant supply of lipid that there was no need to dye it to facilitate observations on adsorption. Enough wax was adsorbed to be clearly evident as an opaque, white layer beneath the translucent, gray masses of unimpregnated lipid (fig. 2, C). On *Eleodes* beetles, large quantities of wax were adsorbed from the undersides of the elytra, but not from the upper sides, except along the suture between the elytra. On *Eleodes*, as well as other insect genera, the wax was always adsorbed in by far the greatest quantities along sutures and at the anterior and posterior margins of abdominal sclerites or at the junctures of body regions. In other areas, there was only enough wax to cover the smallest particles or aggregates of powder.



## Migration of Wax Molecules Over Solid Surfaces

Adsorption, as here used, pertains only to the wax taken up at the interfaces of the silica particles and the wax substrate. The photomicrographs in figure 2 indicated that the wax was able to permeate the entire mass of a silica aerogel agglomerate. Therefore, besides being adsorbed at the interface, the wax molecules must have diffused upward in some manner over the silica surfaces, for distances of up to 30 or 40  $\mu$ .

The adsorption of wax and the movement of wax, or its constituents, over solid surfaces have been not only observed, but also systematically studied in previous investigations. These investigations showed that the mobility of molecules in waxes at room temperature is greater than might be concluded *a priori*.

In a mixture of paraffin wax fractions with a melting point of 69° C. and stearic acid (m.p. 68.2° C.), the surface molecules would normally be regarded as completely fixed, at least within relatively narrow limits. However, Rideal and Tadayon (1954a) demonstrated that when a drop of water was placed on such a surface, its contact angle became progressively lower, changing within a few hours from original values of over 100° to values ranging to approximately 60°, depending on the percentage of stearic acid, which ranged from 0.6 to 100 per cent. This indicated to the experimenters that the stearic acid molecules turned over so as to present a greater number of carboxyl groups to the water phase.<sup>4</sup>

The rate of "overturning" of the stearic acid molecules varied according to the metal substrate on which the monolayers occurred, for the metals had different degrees of "attraction" for stearic acid.

From wax layers on a given metal, stearic acid monolayers could be transferred to another metal to which the molecules were more strongly attracted. The order of attraction of a number of metals was platinum > copper = nickel > tin = aluminum > silver = mica.

It was shown by Rideal and Tadayon (1954b) that stearic acid molecules can be not only "overturned" and anchored to new surfaces, but that they can also migrate along the new surface for a considerable distance. They deposited a C<sub>14</sub>-labeled monolayer of stearic acid over a 1.5-cm. area of mica substrate and determined its rate of diffusion beyond this area, using a direct counting technique. The surface diffusion at 35° C. was found to be  $5.3 \times 10^{-8}$  cm.<sup>2</sup>/sec. Surface diffusion of stearic acid over the first monolayer

<sup>4</sup> Dr. J. J. Bikerman has expressed to the writer the belief that the contact angles in the above experiment decreased because of diffusion of wax into water and its spread over the water surface (Marangoni effect), not because of an overturning of molecules. However, the mobility of molecules within a solid wax is demonstrated by a number of other phenomena, such as "bloom," a powder deposit of wax appearing on the surface of beeswax at ordinary room temperature (Bisson, Vansell, and Dye, 1940). Wax bloom may also be extended from the outer zone of shellaclike material of the insect epicuticle (Wigglesworth, 1957; Richards, 1958).

The rate of lateral movement of Oil-Soluble Red dye from a particle of this powder placed on beeswax is also of interest. The powder dissolves in the wax and spreads from the initial point of contact. The writer has measured the rate of progress of this dye, at 26.7° C., at about 125  $\mu$  per day, a distance that is about 500 times the thickness of the average lipid layer in the insect epicuticle.



of this compound deposited on a substrate was more rapid than that of the stearic acid comprising the monolayer itself. This may explain the deep color of the individual strands of silica aerogel aggregates resting on dyed wax in the present investigation, indicating deposits of much more than a monolayer of wax.

Professor W. F. Seyer, of the Department of Engineering, University of California, Los Angeles, informs us that if a piece of paraffin wax is placed on a clean metal, a layer will migrate over the metal at room temperature in sufficient quantity to lubricate it.

The phenomenon of migration of solids over solid surfaces is further illustrated by the migration of benzophenone over surfaces of mica, glass, silver, and polished quartz (Volmer, 1932), and the rapid penetration of insecticide, from large crystals, into dried mud blocks (Hadaway and Barlow, 1951).

It may be stated in general that molecules, atoms, and ions, present on the surface of a solid and not belonging to its lattice, can move along the surface (Bikerman, 1958), and the molecules comprising solid matter are no exception. In the case of waxes and crystals, the only factors that might be a source of surprise, to those not familiar with the phenomenon, are the speed and the extent of the process.

### Physical Characteristics Affecting the Ability of a Powder to Remove Wax and to Kill Insects by Desiccation

In table 1 are listed, for sixteen powders, those physical characteristics that *a priori* might be assumed to affect the ability of these powders to remove wax from the insect epicuticle and thereby cause death of the insect by desiccation. The powders were selected to present a wide range of such characteristics as particle size, pore size, specific surface, and abrasiveness. An attempt was made to correlate these characteristics with (1) the ability of the powders to remove beeswax from plastic vials, and (2) the ability to knock down or kill four species of insects.

With regard to table 1, the data on pore diameters, specific surfaces, and to a large extent the particle sizes, were obtained from the manufacturers of the powders or from industrial testing laboratories, although some of the particle sizes were determined by the writer.

In order to determine the relative abrasiveness of the powders, pieces of vinyl plastic, of the type used for portfolio covers, were placed in a flat-bottomed glass bowl and covered with powder. The powder was then rubbed onto the plastic sheets in a uniform manner by means of a No. 8 rubber stopper. This was continued for one minute on each side of each sheet. Light from a microscope lamp was passed through the abraded area of each plastic sheet and recorded for intensity, five inches from the sheet, by means of an exposure meter with cover closed. The deviation of the exposure meter reading from that recorded from an unabraded sheet of the vinyl plastic indicated the number of foot-candles of light not reaching the exposure meter because of the increased opacity of the plastic sheet caused by the abrasion. This number was termed the "abrasive index."



TABLE 1

RELATION OF PARTICLE SIZE, PORE SIZE, SPECIFIC SURFACE, AND ABRASIVENESS OF SIXTEEN POWDERS TO  
BEESWAX ADSORPTION AND KNOCKDOWN OF FOUR SPECIES OF INSECTS\*

Powder	Average particle size	Average pore diameter (angstroms)	Average specific surface (meters <sup>2</sup> /g.)	Abrasive index†	Beeswax adsorption (mg./50 cm. <sup>2</sup> )		Decreased opacity of wax films (foot-candles)	Period for 100 per cent knockdown or mortality (hours)			
					Motionless 24 hours	In motion one hour		<i>Drosophila</i> (knock-down)	German cockroach (knock-down)	Drywood termite (mortality)	<i>Tribolium</i> beetle (mortality)
Silica aerogel SG-68.....	82% 0.1 — 3.2 $\mu$	115	300	0	11.0 $\pm$ 1.1	6.1 $\pm$ 0.7	11.6 $\pm$ 1.6	1.9	0.97	3.1	22
Silica aerogel SG-67.....	82% 0.1 — 4.8 $\mu$	115	247	0	12.5 $\pm$ 1.2	5.2 $\pm$ 0.4	14.4 $\pm$ 1.9	1.6	0.65	3.1	17
Silica gel AL-1.....	Average 10.0 $\mu$	22	700	0	20.1 $\pm$ 1.0	8.3 $\pm$ 0.8	18.4 $\pm$ 1.9	1.5	0.03	1.7	29
Silikel.....	85% 1-5 $\mu$	122	36	0	3.2 $\pm$ 0.36	1.3 $\pm$ 0.21	6.8 $\pm$ 1.0	3.0	5.4	9.6	162
Activated carbon.....	90% 325-mesh	335‡	545	1	15.9 $\pm$ 1.3	7.8 $\pm$ 0.7	.....§	1.0	0.93	1.7	24
Olancha Clay.....	91% 325-mesh	22	130	2	12.2 $\pm$ 1.0	7.1 $\pm$ 0.8	12.2 $\pm$ 0.8	1.6	1.25	3.2	25
Attaclay.....	85% 325-mesh	28	120	2	9.2 $\pm$ 0.4	6.7 $\pm$ 0.5	10.6 $\pm$ 0.7	2.1	1.70	3.8	72
Insecticide Grade Pyro- phyllite.....	93.5% 325-mesh	0	0.45	7	1.0 $\pm$ 0.1	0.7 $\pm$ 0.1	5.2 $\pm$ 0.5	8.0	26.0	25.0	264
Barytes.....	99.7% 325-mesh	0	1.0	1	0.0¶	0.0¶	0.0**	6.8	22.6	26.0	216
Molecular Sieves 5A.....	Average 2.7 $\mu$	5	700-800	22	3.8 $\pm$ 0.3	.....††	6.6 $\pm$ 1.0	3.2	3.5	12.4	168
Molecular Sieves 13X.....	Average 2.7 $\mu$	13	700-800	17	6.5 $\pm$ 0.7	.....††	10.8 $\pm$ 1.6	2.4	1.2	9.3	144
Sand.....	100% 200-mesh	0	?	20	0.0¶	0.0¶	2.0 $\pm$ 0.8	14.7	72.0	78.0	264
Carborundum 100.....	90% 100-mesh	0	?	22	0.0¶	0.0¶	0.3 $\pm$ 0.3	33.0	114.0	628.0	312
Carborundum 240.....	95% 200-mesh	0	?	22	0.0¶	0.0¶	0.8 $\pm$ 0.7	24.0	96.0	161.0	252
Carborundum 400.....	79% 5-19 $\mu$	0	?	20	0.0¶	0.0¶	1.8 $\pm$ 1.0	10.8	43.0	45.0	224
Carborundum 600.....	85% 1-10 $\mu$	0	?	15	0.8 $\pm$ 0.07	0.3 $\pm$ 0.03	2.4 $\pm$ 1.3	5.2	21.3	23.0	193

\* Temperatures during the biological tests ranged from 20° to 29° C.; relative humidities from 25 to 32 per cent. The temperature at the beginning of the experiment was 24° C.; relative humidity, 43 per cent. Temperatures during the beeswax adsorption tests were constant at 26.7° C.

† Decrease in foot-candles of light passing through vinyl plastic sheets abraded with the powder for one minute.

‡ Fifty per cent of the volume of the powder was made up of pores greater than 320-350 angstroms in diameter.

§ A minute film of this black powder could not be removed by washing and tended to decrease transparency.

¶ Data for the montmorillonite clays in general. No specific determination has been made for Olancha Clay.

\*\* Some of the particles became embedded in the wax, resulting in a net gain in weight of the wax film. Since these powders are practically nonporous, the particles that became embedded in the wax resulted in a slight net gain in weight.

†† The incorporation of Barytes into the wax film was so conspicuous as to be easily visible with the unaided eye and resulted in an increased opacity of 4.6  $\pm$  0.7 foot-candles.

‡‡ Molecular Sieves reach high temperatures when agitated, resulting in the softening of the wax.



To determine the relative abilities of the powders to adsorb beeswax, plastic vials 2.5 cm. wide and 7.5 cm. long were dipped into melted white beeswax to form films of wax about  $65\ \mu$  in thickness and with an area of about 60 sq. cm. The vials, with lids, were placed in eight-ounce jars and covered with powder. Some were left standing for twenty-four hours, and others were turned end over end at the rate of 108 revolutions per minute, with a mechanical device, so that the powder would be in constant motion against the wax. This agitation was continued for one hour. At the end of each of these twenty-four-hour or one-hour periods, the vials were swabbed with cotton under running water. They were then air-dried and weighed, in order to determine the loss of weight caused by the adsorption of wax. Five vials were used for each of the sixteen powders tested. Five waxed vials, serving as checks, were not placed in powder, but were washed and weighed like the others. Weight lost by these vials was subtracted from that which was lost by the vials that had contact with powder.

With each decrease in the thickness of a wax layer, there should be a corresponding decrease in the opacity of the film. To test this assumption, glass microscope slides were coated on both sides with  $0.4\text{-}\mu$  films of white beeswax. These were placed in various powders for six twenty-four-hour periods, after each of which the powders were removed, as previously described for the waxed vials. Five slides were used for each powder. The slides were then placed before a source of electric light, and the amount of light passing through the wax was determined by means of an exposure meter at a constant distance from the slides and in a fixed position.

### The Action of Various Powders Against Insects

The pertinent physical characteristics of the powders having been determined, the next procedure was to determine their action against four species of insects. The test insects used were adult *Drosophila pseudoobscura*, adult males of the German cockroach (*Blattella germanica*), fully developed nymphs of the drywood termite (*Kaloterme minor*), and adults of the confused flour beetle (*Tribolium confusum*).

One cubic centimeter of the powder was placed in a four-ounce jelly jar with a bottom area of 45 sq. cm., and the insects were placed in the powder in the following numbers: *Drosophila*, approximately fifty (by aspiration from a mixing jar); cockroaches, five; termites, ten; and *Tribolium*, ten. All tests were made in triplicate. After the insects were placed in the jars, the latter were shaken so as to cover each insect completely with a film of powder. An excessive quantity of powder was used, in order to eliminate variables that could be attributed to inadequate or uneven deposit of powder such as might result if minimal quantities were used. It should be borne in mind, however, that if the quantity of powder per unit area is decreased beyond a certain point, the differences in the insecticidal effectiveness of powders of different bulk densities are greatly accentuated. The heavier powders may not provide a layer of sufficient bulk to enable the insects to develop a cloud of dust about their bodies or otherwise pick up a lethal quantity. For example, when SG-67 and Olancha Clay were used at the excessive quantity of 0.42 cc. per 6.5 sq. cm., the latter, which is 5.3 times greater in bulk

density, required 3.1 times longer to result in a 100 per cent knockdown of cockroaches by desiccation. However, when both powders were used in a thin film at only 1.5 mg. per 6.5 sq. cm., Olancha Clay required 12.6 times longer than SG-67 to give a 100 per cent knockdown.

In table 1, the only properties of the powders that are generally correlated with insecticidal effectiveness are their specific surfaces, their pore sizes, and their wax sorptivities. Although the specific surface of a powder is generally correlated with insecticidal effectiveness, this is not necessarily the case, for a powder may have a large specific surface but have pores that are too small to admit the large wax molecules. This is clearly the case with the Molecular Sieves. The two types used in these experiments, 5A and 13X, have uniform pores of 5 Å and 13 Å, respectively, in diameter. As the name implies, Molecular Sieves are designed to allow molecules of a certain size to pass through and to "sieve" out larger ones. For example, type 5A can adsorb propane and higher *n*-paraffins up to at least C<sub>14</sub>, but not any of the *iso*-paraffins. Judging from the period required for perfectly dry Molecular Sieves 13X to knock down cockroaches, it is about equal to Olancha Clay in its ability to adsorb the epicuticular lipid of this insect. According to Beaumont (1955), the liquid solvent that gives cockroach lipid its mobile character may be about equal in quantity to the solid wax and probably consists of paraffins and alcohols of comparatively short chain lengths of the order of C<sub>8</sub>-C<sub>12</sub>. Such molecules could undoubtedly be adsorbed by the Molecular Sieves in sufficient quantity to result in a substantial increase in the intramolecular spacing of the remaining hard-wax molecules and a consequent passage of water through the epicuticle. However, Molecular Sieves 13X is inferior to Olancha Clay in its ability to adsorb the much harder beeswax, which contains few molecules below C<sub>30</sub>. It is also inferior to Olancha Clay in its ability to kill insect species other than the cockroach, which possess wax of much higher average molecular dimensions.

On the other hand, a powder may have pores of adequately large diameters but may nevertheless have a small specific surface. An example of such a powder in table 1 is Silikil, which has a low wax sorptivity and relatively low insecticidal effectiveness, despite having large pores.

As shown in table 1, the silica gel AL-1 has a spectacular insecticidal effectiveness when it is dry. Cockroaches are knocked down in two minutes. However, a serious drawback in the use of both AL-1 and Molecular Sieves is that they have an especially high affinity for water. Therefore, they deteriorate rapidly, in their ability to adsorb insect wax and desiccate insects, when the dry powder is taken from a sealed can and exposed to air for a few hours, for they adsorb too much atmospheric moisture. For example, the manufacturers state that silica gel AL-1 adsorbs 24 per cent of its weight in water at 40 per cent relative humidity, 40 per cent at 80, but only 42 per cent at 100. In tests made by the writer, among desiccated powders allowed to remain at 60 per cent relative humidity for two weeks, AL-1 adsorbed 27.4 per cent of its weight in water and Molecular Sieves 13X adsorbed 29.3 per cent.

Insects are killed by dehydration when they have lost approximately half their water content, amounting to about a third of their body weight (Ebel-



ing and Wagner, 1959). Knockdown can occur long before this point is reached, however, depending on how rapidly the dehydration takes place. The rapid knockdown of cockroaches by the silica gel AL-1 is a measure of the rapid rate of water loss caused by this powder. Apparently, the insects were put into a state of shock about a half hour before they had suffered a sufficient loss of water to result in death. Normally, the dehydration caused by sorptive powders is not sufficiently rapid to cause a distinct knockdown. The activity of the insect declines gradually until death occurs. This is in contrast to the action of toxicants, which usually cause a rapid knockdown, but the period required to kill the insect is usually greater than that which is required when the same species is treated with the best sorptive powders (Ebeling and Wagner, 1959; Tarshis, 1959).

With the exception of AL-1 and Molecular Sieves, the powders shown in table 1 do not decrease to any important degree in their insecticidal effectiveness, regardless of the period during which they are exposed to air at ordinary humidities. The silica aerogel SG-67 adsorbs no water at 40 per cent relative humidity, 5 per cent of its weight at 80, and 80 per cent at 100. Thus, it retains excellent ability to adsorb wax up to nearly 100 per cent relative humidity. Then, in a water-saturated atmosphere, apparently the fluoride in this powder becomes highly effective against insects and mites (Ebeling and Wagner, 1959; Micks, 1960; and Tarshis, 1960, 1961). The same powder, without the fluoride, acts very slowly in a water-saturated atmosphere.

It appears (table 1) that a pore diameter of about 20 Å is the lower limit for effective wax adsorption, provided the powder has not had a chance to adsorb much atmospheric moisture.

Table 1 shows that all powders that were highly abrasive (Molecular Sieves, pyrophyllite, sand, and carborundum) were poor wax adsorbents and were also ineffective against the insects. There is nothing about abrasiveness *per se* that would decrease the ability of a powder to remove insect wax, but it happens that the abrasive powders are the most apt to be nonsorptive.

Barytes, also an insecticidally ineffective powder, is practically nonabrasive, but it is also nonsorptive and therefore relatively ineffective for killing insects by desiccation.

It will be noted that the *Tribolium* beetle, believed by some investigators to require an abrasive powder because of its protective tectocuticle, was relatively resistant to the action of sorptive powders. However, it required an average of only 13.9 per cent as long to obtain a 100 per cent kill of this beetle in sorptive powders as in the nonsorptive ones. With the exception of Barytes and Silikil, the nonsorptive powders were highly abrasive. On the average, *Tribolium* beetles treated with the abrasive powders died only 21.5 per cent more rapidly than those left in untreated containers.

Table 1 also shows that carborundum increased in insecticidal effectiveness with diminishing particle size. This confirms the results of David and Gardiner (1950), who ascribed the greater effectiveness of the finer powder to its greater ability to abrade the cuticle. When comparing 700-mesh carborundum (very fine) with 150-mesh (coarse), these investigators found that only the finer dust caused abrasion of the beetle *Rhizopertha*. They used the am-

moniacal silver nitrate staining technique of Wigglesworth (1945) for revealing the abraded areas. Except for the tips of the appendages, abrasion was noted only where two parts of the body moved against each other, such as the mouth parts, leg joints and sides of the abdomen, and under the margins of the elytra.

David and Gardiner (1950) followed Wigglesworth (1944, 1945, 1947a) and others in attributing lethal action to the abrasion of the cuticle and consequent water loss. Abrasion can probably account for a low order of insecticidal action in some instances. However, the specific surface of any powder greatly increases with decreasing particle size, even though the powder be nonporous. A slight ability to adsorb wax was demonstrated with carborundum 600 (table 1). Also, it should be borne in mind that the ability of carborundum to adhere to an insect may increase as much as 31-fold with decreasing particle size from the "240" to the "600" grade (David and Gardiner, 1950). These factors must be taken into account when speculating on the mode of insecticidal action of the finer grades of carborundum and other nonporous powders.

Barytes increased the weight of wax films to the extent of  $2.4 \pm 0.34$  mg./60 cm<sup>2</sup>. This heavy (but nonsorptive and virtually nonabrasive) powder has a greater tendency than the other powders to incorporate itself into the wax film, even when not in motion over the wax surface. Nevertheless, when compared with the untreated checks, Barytes can be seen to have a certain feeble insecticidal effectiveness, comparable to that of pyrophyllite and the finer grades of carborundum. Besides Barytes, the other heavy and nonsorptive powders (pyrophyllite, Molecular Sieves, sand, and carborundum) had some tendency to incorporate themselves into the wax film. For some of these (sand, carborundum 100 and 400), this resulted in a slight net gain in weight. No doubt all of the above powders adsorbed some wax, and in the case of pyrophyllite and carborundum 600, as well as the porous Molecular Sieves, this resulted in a net loss in weight of wax. The sorptive ability of a powder appears to be more satisfactorily indicated by the resulting decrease in opacity of the wax film. As shown in table 1, all powders except Barytes increased the amount of light passing through a beeswax film.

The increase in the opacity of the glass slides caused by the double layer (total of  $0.8 \mu$ ) of beeswax averaged about 48 foot-candles. This maximum opacity was decreased by the six successive adsorptions in the various powders to the extent shown in the table (averages for the five slides). When these figures are compared with the quantities of beeswax adsorbed, also shown in table 1, they indicate that the ability of powders to decrease the opacity of wax films is approximately proportional to their ability to remove wax, as indicated by gravimetric analysis. This, in turn, is determined primarily by the adsorbency of the powders.

The above experiment confirms the findings of Helvey (1952) in tests with the larvae of the Mexican bean beetle, *Epilachna varivestis*. His electron micrographs showed that the powders with little or no insecticidal value had particles containing sharp edges and points, and that the most effective powders were also the least abrasive.

Alexander *et al.* (1944) found that inert finely divided powders increased



in their ability to kill insects as they decreased in particle size down to the optimum size of about 1  $\mu$ . Nevertheless, in the present experiments, the highly abrasive Molecular Sieves, with an average particle diameter of 2.7  $\mu$ , and 600-mesh carborundum, with 85 per cent from 1 to 10  $\mu$ , were inferior in insecticidal effectiveness to sorptive 325-mesh powders such as activated carbon, Olancha Clay, and Attaclay, which have an average particle-size range of from 15 to 18  $\mu$ .

As stated previously, some sorptive powders have the ability to adsorb wax at 100 per cent relative humidity, and cause the insects to lose water. Silica aerogels appear to be particularly effective in this connection. In one experi-

TABLE 2  
WEIGHT LOSS AND MORTALITY OF GERMAN  
COCKROACHES LEFT FOR TWENTY-FOUR  
HOURS IN VARIOUS POWDERS AT 100  
PER CENT RELATIVE HUMIDITY\*

Powder	Weight loss (per cent)	Mortality (per cent)
SG-68.....	41.7	100
Olancha Clay.....	24.8	46.7
Activated carbon.....	25.5	53.3
AL-1.....	9.4	0.0
Molecular Sieves 13X.....	7.0	0.0
Carborundum 600.....	2.8	0.0
None.....	1.3	0.0

\* The powders were left for eighteen hours at 100 per cent relative humidity before the insects were added. The temperature was 24.5° C. while they were in the powder.

ment, six powders were placed in four-ounce jelly jars and left for twenty-four hours at 100 per cent relative humidity. The humid chambers were glass desiccators with an inside diameter of 20.3 cm. and containing 720 ml. of distilled water. Then, for each powder, five male German cockroaches were placed in each of three jars; the jars were briefly shaken to insure a complete film of powder on each insect, and left in the humid chamber. Table 2 shows the per cent loss of weight and per cent mortality of the cockroaches after they had been left in the powder for twenty-four hours. Powder adhering to their bodies was removed with a camel's-hair brush before they were weighed. Experiments with freshly dusted cockroaches showed that the brief period required for cleaning and weighing the insects in the ambient atmosphere was not sufficiently long to account for any significant weight loss.

The above experiment might be subject to the criticism that the removal of the cover at the time the cockroaches were placed in the desiccator temporarily decreased the relative humidity in this chamber below 100 per cent. Therefore, an experiment was made in which it was not necessary to remove the cover, for a hole was bored in the rubber stopper at its apex. This hole was normally taped over, but a forceps could be extended through it so as to effect certain manipulations without removing the cover.

Silica aerogel SG-68 was placed in a jelly jar of 7.5 cm. diameter at the usual rate of 1 cc. per 45 sq. cm. This jar also contained an upright vial in which were ten male German cockroaches, confined by means of a gauze cover

held in place with a rubber band. Another vial with ten cockroaches was placed in the desiccator, but outside the jelly jar. After the SG-68 and the cockroaches had remained in the jar for twenty-four hours, the cockroaches were liberated by means of the forceps and crawled about in the powder. Twenty-four hours later, these cockroaches, as well as the check insects, were removed from the desiccator, cleaned, and weighed. The insects in the SG-68 had lost 42.5 per cent of their weight, and the check insects, 3.3 per cent. In an adjoining desiccator in which the same experiment was made except that the lid was removed to place the insects into the desiccator, the cockroaches in SG-68 lost 42.3 per cent of their weight, and the check insects, 3.4 per cent. In both cases, the insects left in the powder were all dead. This indicated that the momentary removal of the lid of the desiccator resulted in no significant difference in the rate of desiccation of the insects. During this experiment, temperatures ranged from 23.3° to 26.7° C.

Insects can lose water at 100 per cent relative humidity only when they are left in continuous contact with the powder. Apparently, water is released from the insect cuticle, even in a water-saturated atmosphere, if the protective wax layer is removed. However, this water must be continuously sponged away by the powder in order that more water may be released from the cuticle. It was shown by Ebeling and Wagner (1959) that when drywood termites were shaken in a sorptive powder, so as to remove their lipid, and the powder was then removed, they did not die in a water-saturated atmosphere. However, they all died within one day if exposed to normal conditions of relative humidity. If left in the water-saturated environment for four days, then placed in the ambient atmosphere, they all survived, having had time to restore the wax removed by the powder.

The ability of certain powders to cause insects to lose water at 100 per cent relative humidity is of great academic interest, but of little or no practical value. However, the addition of a monomolecular layer (4.7 per cent) of ammonium fluosilicate to SG-68 results in the product referred to in this investigation as SG-67. This powder kills cockroaches and fleas about as rapidly at 100 per cent relative humidity as it does under a wide range of humidities, down to as low as 20 per cent. It is believed that the silica continues to remove wax, even in a water-saturated atmosphere. This would allow the fluoride to contact the water-bearing protein layers below the protective lipid and become toxic to the insect, even though normally it cannot be used as a contact insecticide.

### Rate of Wax Adsorption with One Exposure

The inner walls of four glass adsorptive tubes were coated with white beeswax by means of a hot 50 per cent solution of the wax in carbon tetrachloride, containing 1 per cent Oil-Soluble Red dye. Twenty-four hours later, the tubes were placed in a Klett-Summerson photoelectric colorimeter, and readings were made. Then silica aerogel SG-67 was poured into the test tubes and emptied by tapping. This left a film of powder on the wax surface. Colorimeter readings were then made in one, four, eight, and forty-four hours. The increases in the readings resulting from the silica aerogel alone, immediately after application, were subtracted from subsequent readings. The results are shown in figure 3.



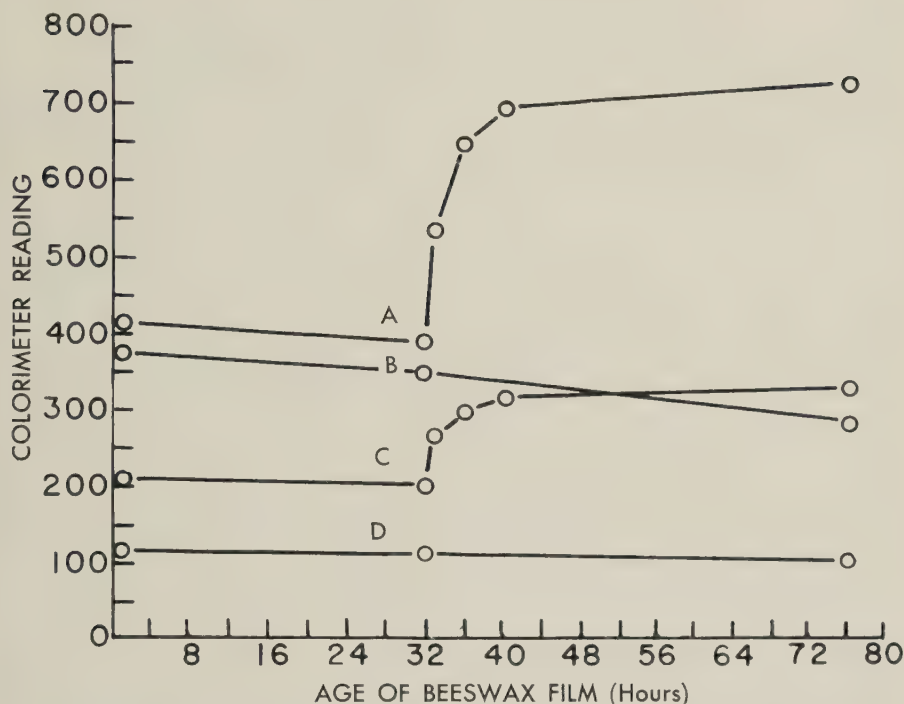


Fig. 3. Net gain in optical density of dyed beeswax-silica aerogel layers after contact of films A and C with silica aerogel SG-67 for thirty-two hours, indicating rate of adsorption of wax with a single exposure to the sorptive powder. The optical density reading resulting from the powder itself was subtracted from the total.

Within one hour, there occurred a sharp increase in the opacity of the wax-silica aerogel layers. This was believed to be caused by the adsorption of the dyed wax by the silica particles, resulting in a scattering of light with a consequent increase in the optical density readings on the colorimeter. Before adsorption, the dyed wax had existed in translucent films with a minimum of opacity.

Within forty-four hours, the colorimeter readings appeared to have reached approximately their maximum values, indicating that about all the wax that could be adsorbed with a single layer of powder had already been removed. However, 46 per cent of the maximum adsorption had occurred in one hour, 76 per cent in four hours, and 88 per cent in eight hours.

### Rate of Wax Adsorption with Successive Exposures

It was believed that successive exposures of the same wax layer or film to fresh portions of sorptive powder might reveal information on the nature of wax adsorption that could not be ascertained by the exposure of the wax to only one application of the powder. Plastic vials 2.5 cm. wide and 7.5 cm. long were dipped into melted yellow beeswax, and others were dipped into paraffin wax, so that layers of wax were formed on their outer surfaces. The layers of wax were about  $65 \mu$  thick on the vials dipped into beeswax and about  $75 \mu$  thick on those dipped into paraffin wax (Parowax). The vials were then weighed, and

half of them were coated with the silica aerogel SG-67, after which the excess powder was removed by light tapping, and the vials were then kept at room temperatures ranging from 23° to 27° C. (average, 24.4° C.) or in an oven at a constant temperature of 48° C. The other half were kept under the same temperature conditions but not in contact with powder. In this and the remaining experiments discussed in the present paper, three vials were used for each test.

At twenty-four-hour intervals, the vials were swabbed with cotton under a stream of cold water. They were then air-dried and weighed, in order to determine the loss in weight caused by the adsorption of wax. Loss of weight with untreated cheeks was extremely small but was nevertheless subtracted from the loss with treated vials. By wiping the inside of the vial with a clean cloth just before weighing, variation caused by differences in degree of moisture sorption on the inner (unwaxed) surfaces of the vials was eliminated.

The results are shown in figure 4. The loss of weight of beeswax at 24.4° C. or at 48° C. (fig. 4, *A* and *C*), or of paraffin at 24.4° C. (*B*), was negligible. However, both dusted beeswax at 24.4° C. (*D*) and dusted paraffin at 24.4° C. (*E*) were readily adsorbed by silica aerogel. The amount of wax adsorbed in sixteen days was about 5.5 times greater for dusted beeswax in contact with silica aerogel at 48° C. (*F*) than at 24.4° C. (*D*).

The weight-loss curve for paraffin at 48° C. (*G*) would not have been so great during the first four or five days if it had not been for the fact that some of the wax softened enough to flow off the vials to the bottom of the container in which the vials were standing in a vertical position. This was not the case, however, when the wax film was covered with silica aerogel (*H*), for the wax was adsorbed so rapidly that it did not flow.

Plotting the loss in weight of wax films at 48° C., or wax films in silica aerogel at either 48° C. or room temperature, resulted in all cases in an exponential curve, as one would expect from first-order reaction kinetics. This is the type of curve that characterizes the selective removal of certain kinds of molecules, which constitute a progressively lower percentage of the remaining mass with each successive removal.

Figure 5 shows the loss in weight of waxed vials placed in eight-ounce jars that were half full of powder and then agitated mechanically, as mentioned previously, for successive periods of one hour, using a fresh batch of powder each time. After each period, the vials were washed and weighed as in the experiments depicted in figure 4. Five of the vials were agitated moderately and uniformly, but the sixth, indicated by the words "severe abrasion" in figure 5, was shaken with greater force and rapidity. The uppermost of the two curves for carborundum depicts the more severe abrasive action.

With carborundum, the first weighing of a series must be rejected because of a gain in weight caused by particles lodged in the wax which are not removable by the washing technique used in these experiments. The quantity appears to be compensable, however, for after the first washing, the wax film underwent a continual reduction in weight as a result of the successive abrasions.

As in the experiment depicted in figure 4, the curves in figure 5, showing the amount of beeswax removed with successive shakings of the waxed vials,



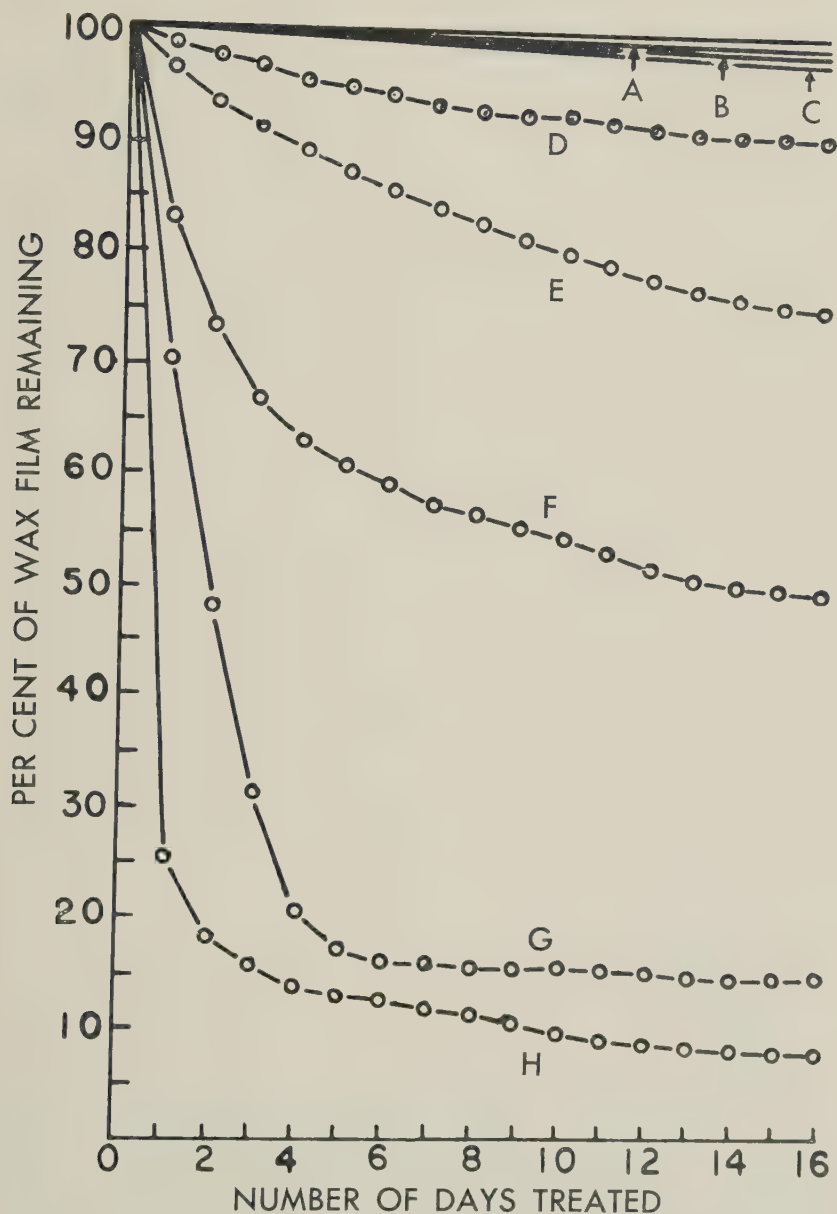


Fig. 4. Per cent decrease in weight of films of beeswax and paraffin on plastic vials, half of which were dusted with silica aerogel SG-67. All vials remained at 24.4° C. or 47.8° C. for twenty-four-hour periods. A, beeswax at 24.4° C.; B, paraffin at 24.4° C.; C, beeswax at 48° C.; D, dusted beeswax at 24.4° C.; E, dusted paraffin at 24.4° C.; F, dusted beeswax at 48° C.; G, paraffin at 48° C.; H, dusted paraffin at 48° C.

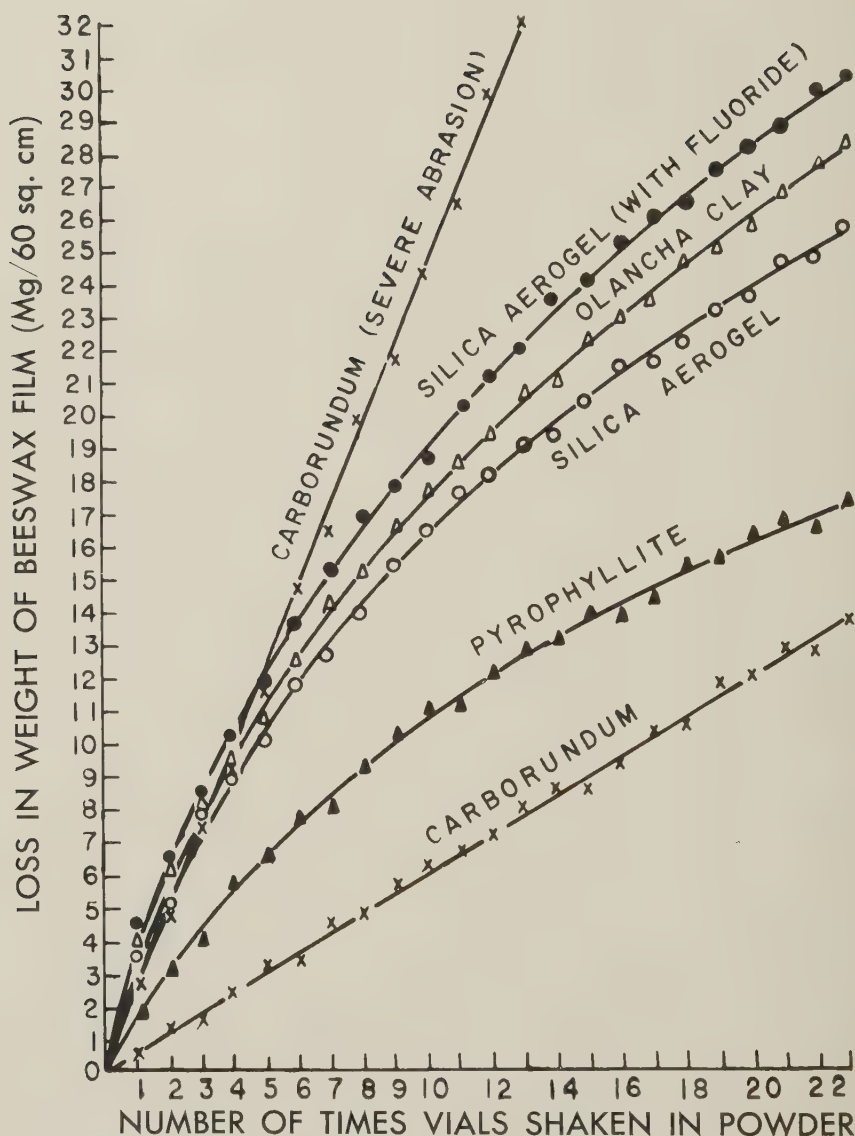


Fig. 5. Weight losses of 65- $\mu$ -thick beeswax films on plastic vials repeatedly immersed in various powders and simultaneously mechanically shaken for periods of one hour, except for the one marked "severe abrasion," which was shaken in a more violent manner, separately from the others.

were exponential in character whenever the removal of the wax was caused primarily by adsorption. With carborundum, however, the beeswax was removed from the vials primarily by abrasion. This was indicated by the opaque and dull appearance of the wax, in sharp contrast to the smooth and translucent appearance of the films on the vials shaken in the relatively



nonabrasive powders. The appearance caused by the carborundum is characteristic of the action of abrasive powders when their particles have been in motion against the wax film. Powders whose action is predominantly adsorptive do not change the appearance of the wax film, even though their ability to remove wax is greater than that of the abrasive powders.

The points representing weight loss of wax from shaking the waxed vials in carborundum form straight lines. This indicates that abrasive powders are nonselective, removing the wax molecules indiscriminately, layer after layer, until all the wax is removed. On the other hand, powders that are primarily sorptive remove only those molecules which are amenable to removal by sorption. Plotting of the weight loss of the wax film when such powders are used, whether the particles of the powder are moving or motionless, invariably results in an exponential curve reaching an asymptote. Regardless of the thickness of the wax film, a point is reached when no more wax can be removed by successive adsorptions, unless the remaining wax is melted or dissolved away with a solvent and redeposited on the vial.

Figure 6 shows the loss in weight of films consisting of white beeswax (*A*) and the yellow beeswax of commerce (*B*) when the waxed vials were shaken in silica aerogel SG-67 for successive ten-minute periods and for one twenty-four-hour period. In this experiment, glass vials 2 cm. in diameter and 8.5 cm. long were dipped in a 3 per cent solution of beeswax in carbon tetrachloride, so that the wax film was  $1\ \mu$  in thickness. A strip of wax about 1 cm. in width was then removed from both ends of each vial, to facilitate handling without touching the wax. It is interesting to note that the adsorption curves reached their asymptote when about 20 per cent (curve *A*) and 24 per cent (curve *B*) of the wax was removed. It was found throughout this investigation that the shorter the successive periods of adsorption, the smaller the ultimate yield of wax. Further removal of wax can be accomplished only by long, continuous contact of the wax film with the powder, whether the powder is in motion or not. This is shown by the curves in figure 6.

Further data on this subject were obtained by immersing waxed glass vials in silica aerogel SG-67 in five successive series of immersions, each consisting of four hourly periods followed by a forty-four-hour period of continuous immersion. No agitation of the vials was involved in this test. The results are shown in table 3. In the first trial, the sum of the quantities of wax adsorbed during the first four hourly immersions was 79 per cent as great as for the following forty-four hours of continuous adsorption. However, the fifth time this comparison was made, the corresponding percentage was 32. Over the ten-day period, the decrease in the yield for four consecutive hour-long adsorptions was 80 per cent, compared with 47 per cent for the forty-four-hour periods of continuous adsorption. The significance of these data appears to be that, as the adsorbed molecules are being withdrawn increasingly farther from the surface with successive adsorptions, the advantage of long, continuous periods of adsorption becomes progressively greater.

Figure 7 refers to an experiment in which glass vials the size of those used in the experiment depicted in figure 6 were dipped in 3 per cent beeswax in carbon tetrachloride, so that the film of wax was approximately  $1\ \mu$  in thickness. The waxed vials were buried in silica aerogel SG-67 for successive

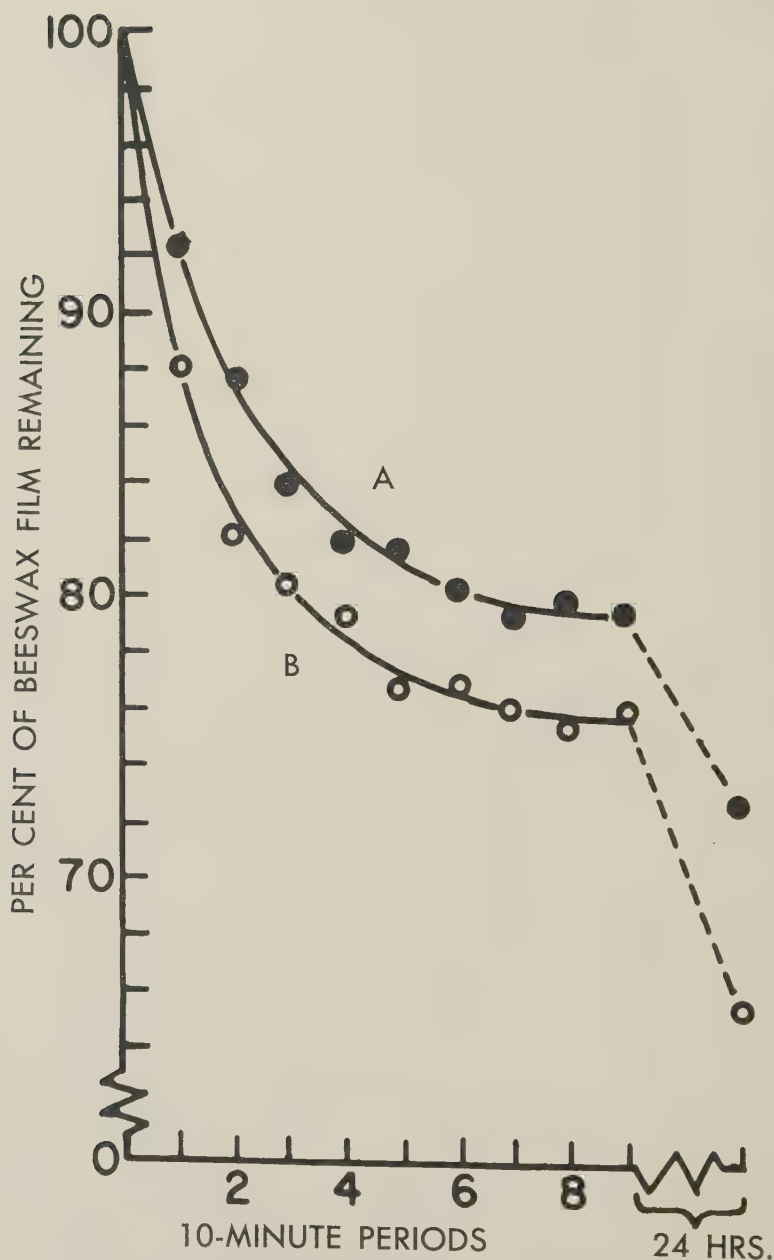


Fig. 6. Per cent decrease in weight of beeswax films on plastic vials repeatedly immersed in silica aerogel SG-67, and mechanically shaken for nine ten-minute periods and one twenty-four-hour period. *A*, white beeswax; *B*, commercial yellow beeswax.



TABLE 3  
DECLINE IN YIELD OF WAX THROUGH ADSORPTION BY  
SILICA AEROGEL SG-67 IN FIVE CONSECUTIVE TRIALS  
OF FOUR ONE-HOUR PERIODS FOLLOWED BY  
ONE FORTY-FOUR-HOUR PERIOD

Consecutive trials	Quantity of beeswax adsorbed (mg.)	
	Four one-hour periods*	One forty-four-hour period†
1.....	2.08	2.62
2.....	1.31	2.44
3.....	0.64	1.68
4.....	0.54	1.47
5.....	0.44	1.39

\* Decrease in four-hour yields, 80 per cent.

† Decrease in forty-four-hour yields, 47 per cent.

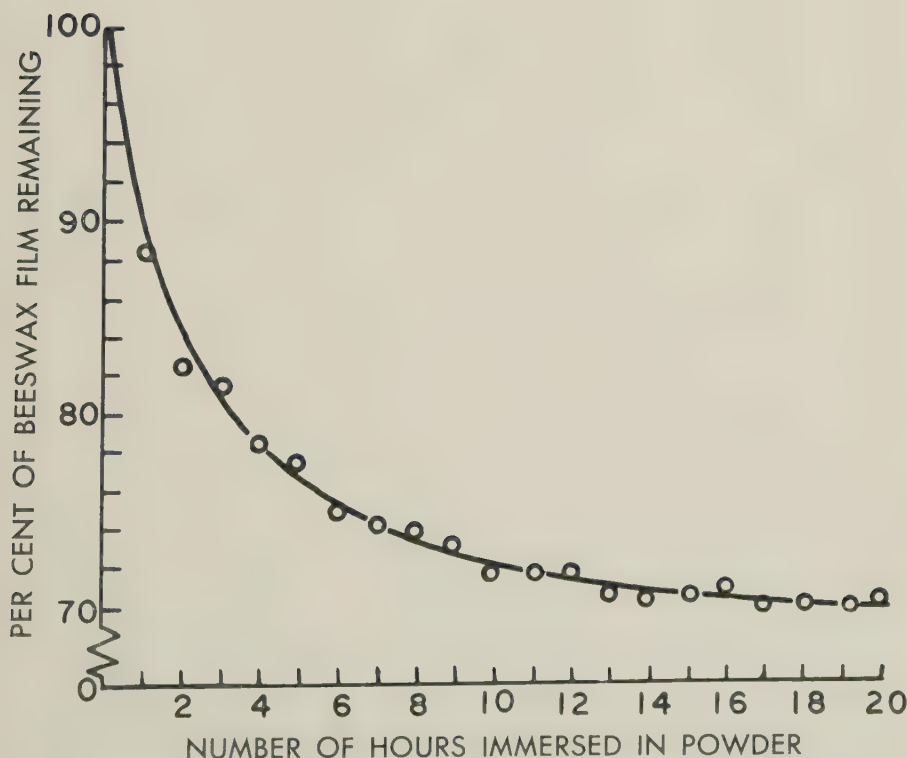


Fig. 7. Per cent decrease in weight of 1- $\mu$ -thick beeswax films on glass vials when repeatedly immersed in fresh silica aerogel SG-67 for periods of one hour.

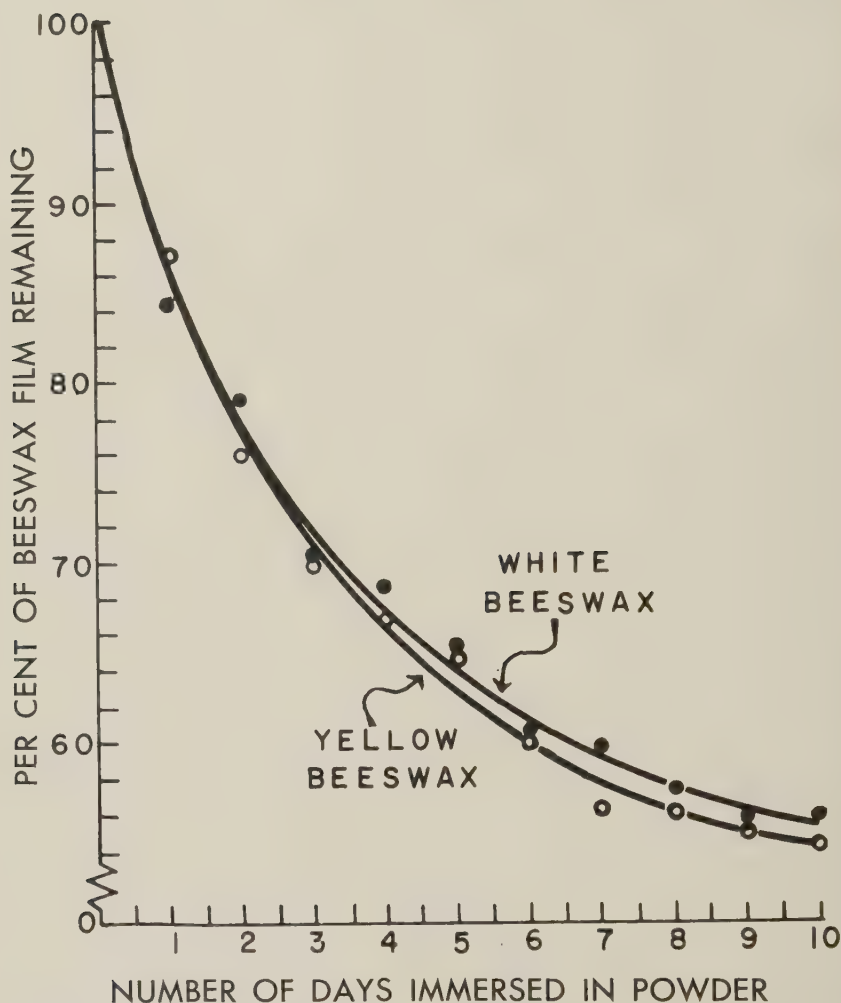


Fig. 8. Per cent decrease in weight of 1- $\mu$ -thick beeswax films on glass vials when repeatedly immersed in fresh silica aerogel SG-67 for periods of twenty-four hours.

periods of one hour. The vials remained motionless, and care was taken to avoid movement of powder against their surfaces. As in previous experiments, the powder was removed at the end of each period of immersion.

The curves in figure 8 should be compared with the one in figure 7. The experiment was made in the same way, except that the waxed vials were immersed in the silica aerogel for twenty-four-hour periods instead of one-hour periods. White beeswax was compared with yellow beeswax, and no significant difference was found. Again the films of wax were 1  $\mu$  thick. Note that figure 7 shows about the same amount of wax adsorbed in four one-hour periods as in the first two twenty-four-hour periods of the experiment shown in figure 8. However, there was no further adsorption of wax after about 29



per cent had been removed. On the other hand, figure 8 shows continued losses of wax from the vials until about 46 per cent had been removed. This again illustrates the necessity for long, continuous periods of adsorption for the highest yields of wax.

Adsorption experiments were also made with much thinner layers of wax, down to a thickness of  $0.32\ \mu$ —well within the range of the thickness of the lipid layer on insects. However, the data were much more variable, and the wax layer had a tendency to flake off in a few places before the experiment had been terminated. Nevertheless, it appeared that about 40 per cent of the wax in these very thin layers could be adsorbed, and the data, despite the greater scattering of points, resulted in curves similar to those shown for  $1\text{-}\mu$  wax layers (figs. 7 and 8).

### Percentage of Wax Removed to Effect Water Permeability

To obtain an estimate of the approximate percentage of wax that must be removed to enable water to penetrate the protective wax layers on insects, glass vials were dipped into a hot 3 per cent solution of white beeswax in carbon tetrachloride. The layers formed were approximately  $0.3\ \mu$  in thickness. Since the C—C distance is  $1.54\ \text{\AA}$ , this figure was used as the basis for calculation of the lengths of typical molecules. The linear component of the distance between the zigzagging carbon atoms can be calculated to be  $1.27\ \text{\AA}$ . Since the acids, hydrocarbons, and the longest branch of the V-shaped esters contain 30–33 carbon atoms, their length was calculated to range from  $36.8$  to  $40.6\ \text{\AA}$ . Assuming the molecules to be unbranched and placed end to end and uncoiled, a  $0.3\text{-}\mu$  layer of beeswax should be between 74 and 81 molecules in depth.

The glass vials with these very thin layers of beeswax were slowly but continuously moved about in revolving jars of silica aerogel SG-67 for thirty minutes. This is the period required to cause a knockdown, by desiccation, of honeybees crawling about in silica aerogel or highly sorptive clay. About 16 per cent of the wax was removed in this period. It appears that some such small percentage of loss of amorphous-wax molecules suffices to make certain insects susceptible to an abnormal and lethal rate of desiccation.

### DISCUSSION

It has been shown by Beament (1945) that the layer of oriented wax molecules resting on the tanned protein of the insect epicuticle provides practically all the protection against water loss. Since the wax must be secreted with sufficient solvent to insure its mobility, it not only can pass through the hardened cuticle during the process of repair, but it is also temporarily in a form readily amenable to orientation. Considering the relative polarities of the components of insect wax (acids, alcohols, esters, and hydrocarbons), one might expect that the first layer of molecules at the interface between the lipid and its hydrophylic substrate might reasonably consist principally of wax acids and alcohols. Since the nonpolar ends of these molecules would extend away from the interface, the next layer of molecules might contain many esters and hydrocarbons.

The oriented layer of molecules must be considered to be closely packed, for the intramolecular spaces are normally impervious to water. Apparently,

the removal of only a small percentage of these molecules enables the relatively small water molecules to pass through the widened intramolecular spaces. The present investigation indicated that, with honeybees, only about 16 per cent of the wax need be removed, and that this may be accomplished in as brief a period as thirty minutes. This would explain the rapid effect of sorptive powders on certain small insects such as *Drosophila*, ants, and hymenopterous parasites. However, merely a disorganization of the layer of oriented molecules could also have the effect of making it penetrable by water.

The physical chemistry of the removal of wax from the insect epicuticle by adsorption must, for the time being, remain speculative. X-ray patterns of white beeswax rodlets, 1 mm. in diameter, showed that the crystallites that remained after eight twenty-four-hour adsorptions with silica aerogel SG-67 were identical to those in the original wax. If the wax consisted entirely of crystallites and these were adsorbed by the silica powder, one would expect that, in the case of a wax film, the entire film could be removed and that the rate of adsorption would be a straight-line function. However, since no more than 46 per cent of the wax was ever removed by adsorption in the present series of experiments, it was concluded that the adsorbed material was amorphous (see p. 536) and that the crystallites could not be removed.

Apparently, when a nonadsorbed wax film is dissolved in a suitable solvent, a random redistribution of molecules occurs, so that, when a new film is formed, approximately the same percentage of amorphous material is again present and amenable to adsorption.

Infrared spectra of beeswax showed a higher peak for wax acids before contact of the wax with silica aerogel than after repeated contacts. It is conceivable that the acids of the amorphous wax might be preferentially adsorbed by the polar silica particles, but since the acids constitute less than 15 per cent of the wax film, this would account for only a small part of the adsorbed wax. It is most likely that the exponential nature of the adsorption curve resulted primarily from the preferential adsorption of the amorphous wax in general. With each successive adsorption, less material was removed from the film, until only the unadsorbable crystallites remained.

As amorphous-wax molecules were removed from the surface layers, those farther down would take their places, and so on down through the wax film. Eventually, minute channels would be formed through the remaining crystallite matrix. In addition, a sufficient number of molecules would be removed from the closely packed and oriented layer at the lipid/protein interface to result in a significant increase in the intramolecular spacing in this layer. This would, in turn, result in the escape of the relatively small water molecules from the water-bearing protein substrate.

It is realized, of course, that in the insect epicuticle the presence or absence of the "tectocuticle," and its consistency or location, are factors that would alter the process of wax removal from the ideal representation afforded by a homogeneous layer of beeswax. However, experience indicates that such factors affect only the speed of the process, rather than its essential mechanism.

With adsorption, it is not necessary to remove all the wax at a given area of the insect's epicuticle in order to cause water loss at that point. As explained above, desiccation begins when only a small fraction of the wax has



been removed. In contrast, with abrasion the wax must be completely removed, down to the water-bearing tanned protein of the insect's epicuticle, in order to cause water loss at a given point.

The epicuticle has no pores visible, even with the aid of an electron microscope (see Richards and Anderson, 1942), but water is copiously supplied to this thin hydrophilic layer (*circa*  $1\ \mu$ ) by means of myriads of pore canals extending through the procuticle. Pore canals commonly have a density of about 1,000,000 per sq. mm. (Richards, 1958). In certain species, these eventually become plugged with cuticular material, but obviously the "solid" procuticle is readily permeable to water. Thus, the mechanism exists for the rapid passage of water out of the insect's body at an abnormal rate under a rather wide variety of conditions: (1) when a certain percentage of the molecules in the wax layer on the epicuticle has been removed by the process of selective adsorption of molecules described in the present paper; (2) by the removal of wax the entire distance down to the lipid/protein interface by abrasion provided by the experimenter, as with an emery cloth, or when the insect's habits are conducive to the abrasive action of dusts; (3) by an increase in temperature to the point at which the oriented molecules of the organized portion of the lipid protective layer are disorganized (Beament, 1958); (4) by the application of oil or solvents (Wigglesworth, 1942, 1945; Hurst, 1943; Beament, 1945; and Ebeling and Wagner, 1959), particularly those with the most suitable balance of hydrophilic and lipophilic characteristics; and (5) by the application of any of a wide variety of insecticides (Ebeling and Pence, 1957; Kitaoka and Yajima, 1958; and Ebeling and Wagner, 1959).

## SUMMARY

Beeswax is adsorbed at room temperature by silica aerogel and other sorptive powders and subsequently migrates from particle to particle to saturate an entire aggregate of powder. This was demonstrated by allowing silica aerogel powder to settle on beeswax layers from a cloud of dust and then observing the change in color of the aerogel from a translucent gray to an opaque white as it gradually adsorbed the wax. The observation of adsorption was facilitated by the use of a dyed beeswax or the dyed cuticles of various species of insects.

A study was made of the correlation of certain physical characteristics of finely divided powders, such as particle size, pore size, specific surface, abrasiveness, and ability to adsorb beeswax, with their ability to cause knock-down or death by desiccation of four species of insects. The only characteristics of the powders that were generally correlated with high insecticidal effectiveness were adequate pore size, large specific surface, and the resulting high degree of wax sorption. Porous powders, even those with a large specific surface, were generally ineffective if their pores were below  $20\ \text{\AA}$  in diameter. Such powders might have a high sorptivity for water and the smaller organic molecules, but their pores appeared to be too small to adsorb adequate quantities of the larger wax molecules (*circa*  $C_{30}$ ) that are characteristic of insect waxes. However, the lipid of the cockroach epicuticle, which contains a large quantity of relatively small molecules of the order of  $C_{15}$ – $C_{18}$ , can be adsorbed in sufficient quantity by perfectly dry Molecular Sieves, with a pore diameter of  $13\ \text{\AA}$ , to result in death by desiccation in seventy-two minutes.

One silica gel (AL-1) resulted in a knockdown of German cockroaches in two minutes. Apparently, these insects lost water at such a rapid rate that they were put into a state of shock about a half-hour before they had lost enough water to result in death.

The above statements refer to dry powders. Some of these adsorb too much water under ordinary atmospheric conditions and do not retain their ability to adsorb wax. Others adsorb very little water until high humidities are reached; these are the only powders that are of practical usefulness in pest control.

The rate of weight loss of wax films on glass or plastic vials from successive adsorptions with powders was an exponential function, indicating that the adsorption was selective, removing only certain wax constituents. This was true, whether the waxed vials were agitated or were allowed to remain completely motionless in the powder. Since X-ray patterns indicated that beeswax was only partially crystalline, it was concluded that amorphous material was preferentially removed.

Infrared spectra indicated a preferential adsorption of wax acids from 1- $\mu$  films of beeswax by silica aerogel powder. However, acids constituted less than 15 per cent of the total, and as much as 46 per cent of the wax was adsorbed. Probably, any molecules in amorphous wax can be adsorbed.

When the remaining wax was redissolved and new films were thus formed, the adsorption of wax by silica aerogel was almost as rapid as before. This indicates that when the remaining layer of crystallites was reorganized by dissolving in a solvent and preparing a new layer, amorphous wax again appeared, and adsorption by means of silica aerogel powder could again be accomplished.

When agitated with carborundum, which is abrasive but nonsorptive, the rate of weight loss of beeswax was a straight-line function, indicating an indiscriminate and nonselective removal of wax.

When glass vials with 1- $\mu$  films of beeswax were allowed to remain in silica aerogel for successive periods of one hour, the weight-loss curve reached an asymptote when about 29 per cent of the wax was adsorbed. When left in silica aerogel for successive periods of twenty-four hours, about 46 per cent of the wax was eventually adsorbed.

Decrease in the opacity of waxed glass slides, when immersed in various powders for six successive twenty-four-hour periods, was approximately proportional to the loss of weight of wax and depended on the adsorbency of the powders.

It is believed that solid wax can be removed from wax films by adsorption of surface molecules unattached to crystal lattices and a progressive displacement of successive layers of similarly unattached molecules as they fill the spaces left by those removed in the layer above. With insects, this process would begin at the surface and proceed progressively downward to the interface of the wax layer and its water-bearing substrate. It appears that, with some insects, when only approximately a sixth of the wax film is removed by adsorption, minute channels are created in the matrix of remaining crystallites. Finally, a sufficient number of molecules are removed from the closely packed and oriented layer at the lipid/protein interface to result in a significant increase in the intramolecular spacing of this layer. This, in turn, allows



for the escape of the relatively small water molecules from the water-bearing protein substrate. The lipid film is transformed, in effect, into a "molecular sieve." The resulting rapid loss of water can cause the knockdown or death of some insects in as brief a period as ten or fifteen minutes.

In one test, German cockroaches lost 41.7 per cent of their weight and suffered a 100 per cent mortality when in contact with an unfluoridated silica aerogel (SG-68) at 100 per cent relative humidity and for a period of twenty-four hours. Weight loss in a water-saturated atmosphere takes place at this rate only if the insects are in constant contact with powder. The water appears to be released by the adsorption of the protective wax layer, even in a water-saturated atmosphere. Apparently, it must be continuously sponged away from the cuticle, by means of the powder, in order to allow for passage of more water.

The addition of a monomolecular layer (4.7 per cent) of ammonium fluosilicate to SG-68 causes the treated powder (SG-67) to be as effective against some insects at 100 per cent relative humidity as it is at normal humidities.

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## LITERATURE CITED

- ALEXANDER, P., J. A. KITCHENER, and H. V. A. BRISCOE  
1944. Inert dust insecticides. *Ann. Appl. Biol.* **31**:143-59.
- AZAROFF, L. V., and M. J. BUERGER  
1958. The powder method in X-ray crystallography. McGraw-Hill Book Co., Inc., New York. 342 pp. *See specifically*: p. 266.
- BARTLETT, B. R.  
1951. The action of certain "inert" dust materials on parasitic Hymenoptera. *Jour. Econ. Ent.* **44**:891-96.
- BEAMENT, J. W. L.  
1945. The cuticular lipoids of insects. *Jour. Exptl. Biol.* **21**:115-31.  
1955. Wax secretion in the cockroach. *Jour. Exptl. Biol.* **32**:514-38.  
1958. Waterproofing mechanism of an insect. *Jour. Exptl. Biol.* **35**:494-515.  
1959. The waterproofing mechanism of arthropods. I. The effect of temperature on cuticle permeability in terrestrial insects and ticks. *Jour. Exptl. Biol.* **36**:391-422.
- BIKERMAN, J. J.  
1958. Surface chemistry. Second edition. Academic Press, Inc., New York. 501 pp.
- BISSON, C. V., G. H. VANSSELL, and W. B. DYE  
1940. Investigations on the physical and chemical properties of beeswax. U.S.D.A. Tech. Bul. **716**:1-24.
- CHIU, S. F.  
1939. Toxicity studies of so-called "inert" materials with the bean weevil *Acanthoscelides obtectus*. *Jour. Econ. Ent.* **32**:240-48.
- COTTON, R. T., and J. C. FRANKENFELD  
1949. Silica aerogel for protecting stored seed or milled cereal products from insects. *Jour. Econ. Ent.* **42**:553.
- DAVID, W. A. L., and B. O. C. GARDINER  
1950. Factors influencing the action of dust insecticides. *Bul. Ent. Res.* **41**:1-61.
- EBELING, W., and R. J. PENCE  
1957. The use of pesticides in the preparation of insects and mites for photography. *Ann. Ent. Soc. Amer.* **50**(6):637-38.
- EBELING, W., and R. E. WAGNER  
1959. Rapid desiccation of drywood termites with inert sorptive dusts and other substances. *Jour. Econ. Ent.* **52**:190-207.
- GERMAR, B.  
1936. Versuche zur Bekämpfung des Kornkäfers mit Staubmitteln. *Zeitschr. angew. Ent.* **22**(4):603-30.
- GLYNNE JONES, G. D.  
1955. The cuticular waterproofing mechanism of the worker honeybee. *Jour. Exptl. Biol.* **32**:95-109.
- HADAWAY, A. B., and F. BARLOW  
1951. Sorption of solid insecticides by dried mud. *Nature (London)* **167**:854.
- HELVEY, T. C.  
1952. Insecticidal effect of inert solid diluents. *Science* **116**:631-32.
- HOCKENYOS, G. L.  
1933. Effect of dusts on the Oriental roach. *Jour. Econ. Ent.* **26**:792-94.
- HOLDGATE, M. W., and M. SEAL  
1956. The epicuticular wax layers of the pupa of *Tenebrio molitor* L. *Jour. Exptl. Biol.* **33**:82-106.
- HUNT, C. R.  
1947. Toxicity of insecticide dust diluents and carriers to larvae of the Mexican bean beetle. *Jour. Econ. Ent.* **40**:215-19.

HURST, H.

1943. Principles of insecticidal action as a guide to drug reactivity-phase distribution relationships. *Trans. Faraday Soc.* **39**:390-411.

1948. Asymmetrical behavior of insect cuticle in relation to water permeability. *Disc. Faraday Soc.* **3**:193-210. *See also*: General discussions, pp. 220-39; *specifically*, pp. 232-35.

KALMUS, H.

1944. Action of inert dusts on insects. *Nature (London)* **153**:714-15.

KITAOKA, S., and A. YAJIMA

1958. Effects of insecticides on ticks. II. Effect on loss of body weight and oviposition of ticks. *Japanese Jour. Appl. Ent. and Zool.* **2**(1)11-16.

MICKS, D. W.

1960. Susceptibility of mosquitoes to silica gel insecticides. *Jour. Econ. Ent.* **53**:915-18.

NAIR, M. R. G. K.

1957. Structure of waterproofing epicuticular layers in insects in relation to inert dust action. *Indian Jour. Ent.* **10**(1):37-49.

PARKIN, E. A.

1944. Control of the granary weevil with finely ground mineral dusts. *Ann. Appl. Biol.* **31**:84-88.

RICHARDS, A. G.

1951. The integument of arthropods. University of Minnesota Press, Minneapolis. 411 pp.

1957. Studies on arthropod cuticle. XIII. The penetration of dissolved oxygen and electrolytes in relation to the multiple barriers of the epicuticle. *Jour. Ins. Physiol.* **1**:23-39.

1958. The cuticle of arthropods. *Ergebnisse der Biologie* **20**:1-26.

RICHARDS, A. G., and T. F. ANDERSON

1942. Electron microscope studies of insect cuticle, with a discussion of the application of electron optics to the problem. *Jour. Morph.* **71**:135-83.

RIDEAL, E. K., and J. TADAYON

1954a. On overturning and anchoring of monolayers. I. Overturning and transfer. *Proc. Roy. Soc. (London)* **A225**:346-56.

1954b. On overturning and anchoring of monolayers. II. Surface diffusion. *Proc. Roy. Soc. (London)* **A225**:357-61.

TARSHIS, I. B.

1959. U.C.L.A. tests with desiccant dusts for roach control. *Pest Control* **27**:16-18, 20, 22, 24, 26-28, 30, 32.

1960. Control of the snake mite (*Ophionyssus natricis*), other mites, and certain insects with the sorptive dust, SG-67. *Jour. Econ. Ent.* **53**:903-8.

1961. Laboratory and field evaluation studies with sorptive dusts for the control of arthropods affecting man and animal. *Exptl. Parasitol.* **11**: February-March.

VOLMER, M.

1932. The migration of adsorbed molecules on surfaces of solids. *Trans. Faraday Soc.* **28**:359-63.

WAGNER, R. E., and W. EBELING

1959. Lethality of inert dust materials to *Kaloterмес minor* Hagen and their role as preventives in structural pest control. *Jour. Econ. Ent.* **52**:207-12.

WARTH, A. H.

1956. The chemistry and technology of waxes. Reinhold Publishing Co., New York. 940 pp. *See specifically*: pp. 76-93.

WIGGLESWORTH, V. B.

1942. Some notes on the integument of insects in relation to the entry of contact insecticides. *Bul. Ent. Res.* **33**:205-19.

1944. Action of inert dusts on insects. *Nature (London)* **153**:493-94.

1945. Transpiration through the cuticle of insects. *Jour. Exptl. Biol.* **21**:97-114.



- 1947*a*. The site of action of inert dusts on certain beetles infesting stored products. Proc. Roy. Ent. Soc. (London), ser. A, **22**:65-69.
- 1947*b*. The epicuticle of an insect, *Rhodnius prolixus*. Proc. Roy. Ent. Soc. (London), ser. B, **134**:163-81.
1957. The physiology of insect cuticle. Ann. Rev. Ent. **2**:37-54.
1958. Abrasion of the insect cuticle by aqueous suspensions of small particles. Nature (London) **181**:779-80.
- ZACHER, F.
- 1937*a*. Eine neue Gruppe von Insektiziden. C. R. du XII Congrès Int. de Zool. (Lisbonne), 1935. Pages 2336-40.
- 1937*b*. Neue Untersuchungen über die Einwirkung oberflächenaktiver Pulver auf Insekten. Zool. Anz. **10**, Supplementband, pp. 264-71.
- ZACHER, F., and G. KÜNIGKE
1931. Untersuchungen über die insektizide Wirkung von Oxyden und Karbonaten. Arb. aus Biol. Reichsans. **18**:201-31.

# RELATION OF LIPID ADSORPTIVITY OF POWDERS TO THEIR SUITABILITY AS INSECTICIDE DILUENTS<sup>1</sup>

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## INTRODUCTION

IT HAS BEEN well established that sorptive powders can cause the death of insects, sometimes with remarkable rapidity, by removing a portion of the very thin layer of lipid, averaging about  $0.25\ \mu$  in thickness, that covers the insect epicuticle and normally prevents desiccation (Alexander *et al.*, 1944;<sup>1</sup> Hurst, 1948; Helvey, 1952; Glynn Jones, 1955; Nair, 1957; Ebeling and Wagner, 1959; Wagner and Ebeling, 1959; Tarshis, 1959, 1960, 1961; and Micks, 1960).

It thus might be assumed that sorptive diluents should increase the insecticidal effectiveness of toxicants by adding their independent effect as lipid removers. However, preliminary tests showed that this is not always the case; with some toxicants, the sorptiveness of the diluent proved to be highly detrimental. Obviously, if an inert powder having the ability to cause the death of an insect is to be less effective as a diluent than one without insecticidal effect, some adverse action must take place when that powder is combined with the toxicant.

The purposes of the present investigation were (1) to determine the independent influence of sorptive and nonsorptive powders on the ultimate effects of the toxicants by applying them to insects for brief periods before the latter were treated with the toxicants; (2) to determine the effects of these same powders when used as diluents for the toxicants; and (3) to find an explanation for the differences in insecticidal effectiveness of toxic dusts that might be caused by the different types of diluents.

## MATERIALS AND METHODS

The insect species used in this investigation were adult *Drosophila pseudoobscura*; adult males of the German cockroach, *Blattella germanica*, and of the brown-banded cockroach, *Supella supellectilium*; full-grown nymphs of the drywood termite, *Kaloterms minor*, and of the dampwood termite, *Zootermopsis angusticollis*.

*Drosophila pseudoobscura* is much larger than the familiar *D. melanogaster* and much more resistant both to the effects of toxicants and to the desiccating effect of sorptive powders. The flies used in the present investigation were obtained from the laboratory of Dr. Carl C. Epling, who rears them in large numbers throughout the year in connection with genetics investigations. The flies are reared in a uniform manner in half-pint milk bottles, and

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<sup>1</sup> See "Literature Cited" for citations, referred to in the text by author and date.

various lots are mixed together and aspirated from a single bottle, to insure representative samples for the tests.

The cockroaches were obtained from the laboratory of Dr. I. Barry Tarshis. They were reared in wide-mouthed gallon jars and thrived best on Purina Dog Chow Checkers. Each jar contained a roll of corrugated paper, to afford the necessary hiding and resting places for the cockroaches.

Drywood termites were obtained in large numbers from the dead limbs of walnut trees. Nymphs tapped from split sections of large limbs were placed on about ten disks of paper toweling that had been fitted into petri dishes. The uninjured insects crawled away from the top sheet of paper to find shelter among the sheets below. Those remaining on top were discarded. The termites could be kept for months in good condition between the sheets of paper, which they used for food. The full-grown nymphs were used in the experiments.

Dampwood termites were obtained through the cooperation of local termite operators.

The classifications of the diluents used, and some pertinent physical and chemical characteristics, are shown in table 1. Classifications as "sorptive" or "nonsorptive," referring to powders used alone or as diluents, were established as follows: Plastic vials with an average outer surface area of 61 sq. cm. were dipped into melted beeswax, to obtain a wax film averaging about  $65\ \mu$  in thickness and about 290 mg. in weight. Twenty-four hours later, the vials were mechanically shaken in 50 cc. of powder for one hour. Five trials were made for each powder. The powder was removed by gentle washing with the aid of a cotton swab, and the quantity of wax removed by the powder was determined gravimetrically. This was done at temperatures ranging from  $22^{\circ}$  to  $24^{\circ}$  C. The powders that adsorbed 3 mg. or more of wax from these vials were considered to be "sorptive" and the remainder to be "nonsorptive." The "sorptive" powders included the silica aerogels, montmorillonite clays, attapulgitic clays, and activated carbon. The "nonsorptive" powders included botanical powders, natural and synthetic oxides of silicon, oxides of iron, carbonates, sulfates, silicates, and volcanic minerals. It happens that this division also marks a striking difference in the ability of the powders to kill insects by desiccation.

Dibrom was initially used as the test toxicant because of a chance observation of the adverse effect of sorptive diluents on this compound. To prepare the diluted toxic dusts, a concentrate dust of 20 per cent Dibrom in Pikes Peak Clay was diluted with the various types of diluents to the required concentration. The results with Dibrom were so striking that a comparative evaluation of a wide variety of toxicants, as affected by their diluents in dust formulations, was considered to be desirable. Of all the toxicants tested, Dibrom and the closely related DDVP proved to be the most adversely affected by sorptive diluents of all the toxicants tested, although this tendency was noted, to a lesser extent, with the other organophosphorous compounds, malathion, parathion, and Dylox.

For the evaluation of the diluents or insecticide dusts, one cubic centimeter of the powder was placed in a four-ounce jelly jar, with a bottom area of 45 sq. cm. and with a screened lid. The number of insects placed in each jar was



as follows: *Drosophila*, about fifty, aspirated from the mixing jar; cockroaches, five; and termites, ten. All tests were made in triplicate, and the data in the accompanying tables are the averages of three tests.

"Knockdown" was considered to be the point at which *Drosophila* or termites were unable to move their entire bodies, even though appendages were still moving, and the point at which cockroaches turned over to lie on their backs. With any insect, "death" was considered to be the condition in which no further movements of the bodies or the appendages could be discerned.

The experiments in which the insects were exposed to sorptive or nonsorptive powders before they were treated with toxicants were made as follows: The insects were placed in the powder, as described above. After a predetermined period of contact with the powder, the latter was sieved through the screens on the jar lids. As much powder as possible was blown off the insects' bodies. They were then placed in a toxic dust prepared with a nonsorptive diluent.

### Pretreatment with Sorptive and Nonsorptive Powders

In the evaluation of the effect of diluents in insecticide dusts, data on (1) their independent action (effect when applied previous to the application of the toxicant), and (2) their action when used as diluents, would appear to be pertinent. Tables 2 and 3 show the effects of pretreatment with sorptive and nonsorptive powders on the effectiveness of Dibrom against *Drosophila* and two species of cockroaches.

In the experiments with *Drosophila* (table 2), flies that were pretreated for ten minutes with highly sorptive powders (Olancha Clay, Pikes Peak Clay, and Santocel C), which are also effective in killing insects by desiccation, increased the average period required for 100 per cent knockdown of the flies 3.3-fold when compared with the average period required after pretreatment with walnut-shell flour, Mississippi Diluent, and blue talc, which are all relatively nonsorptive. The period for 100 per cent knockdown was increased 4.4-fold when the flies were pretreated for twenty minutes, but further pretreatment did not influence the period required for toxic action. The average period required for 100 per cent knockdown after pretreatment with walnut-shell flour, Mississippi Diluent (treated), and blue talc was about the same as when the insects were not pretreated.

With the German and brown-banded cockroaches (table 3), pretreatment with sorptive powders had an effect that was directly opposite to that obtained with *Drosophila*. Pretreatment with the sorptive powders (Pikes Peak Clay and Attaclay) decreased the period required for 100 per cent knockdown of the insects by a 2 per cent Dibrom dust. A ten-minute pretreatment had greater effect than a sixty-minute pretreatment. With sorptive powders, it decreased the period required for a 100 per cent knockdown by 60.6 per cent for the German cockroach and 66.7 per cent for the brown-banded cockroach, when compared with the untreated check. As with the *Drosophila*, the average effect of pretreatment with nonsorptive powders (walnut-shell flour and Insecticide Grade Pyrophyllite) was not significantly different from that with no pretreatment.

TABLE 1  
CLASSIFICATIONS AND PROPERTIES OF SOME COMMON INSECTICIDE DILUENTS AND OTHER POWDERS

Class	Diluent	Particle size	Specific surface (meters <sup>2</sup> /g.)	Oil adsorption* (per cent)	pH	Change in weight of beeswax film† (mg.)
BOTANICALS	Walnut-shell flour	97% 100-mesh	0.4-0.5	56	4-5	+ 1.0 ± 0.12
ELEMENTAL MINERALS	Electric Sulfur	98% 100-mesh	0.375	32	5-6.5	+ 1.8 ± 0.22
OXIDES OF SILICON						
Diatomites	Celite 209	91% < 10 $\mu$	15-25	172	7	- 1.0 ± 0.15
	Dicalite 1G-5	90% 100-mesh	22	155	5.7-7.6	- 0.5 ± 0.10
Tripolites	Silica 400-mesh	99.5% 325-mesh	....	46	9.6	- 2.3 ± 0.43
	Silica DCR	100% 325-mesh	....	31	6.9-7.1	- 0.3 ± 0.09
Synthetics	Hi-Sil 233	Average 0.022 $\mu$	160-175	170	7.3	- 3.7 ± 0.24
	Santocel C	"Typical" 3-5 $\mu$	130-150	300	3.5	- 3.0 ± 0.26
	SG-67 (contains fluoride)	82% 0.1-4.8 $\mu$	247	255	2.6	- 3.5 ± 0.48
	SG-68	82% 0.1-3.2 $\mu$	300	300	7.0	- 4.2 ± 0.53
	Silikil D (contains fluoride)	86% 325-mesh	....	51	...	- 1.3 ± 0.21
OXIDES OF IRON	Red iron oxide	98% < 40 $\mu$ 70% < 0.5 $\mu$	....	25	5.5	- 1.2 ± 0.18
CARBONATES						
Calcium limes	Mississippi Diluent	99% 325-mesh	0.55	18	9.3	+ 1.9 ± 0.37
	Mississippi Diluent (treated)†	99% 325-mesh	0.55	18	9.3	+ 2.3 ± 0.47

SILICATES	Mica.....	Blue Diamond Gypsum.....	99.7% 325-mesh	1.0	14	5.8	+ 1.8 ± 0.13
	Talc.....	Calcined gypsum.....	76.4% 325-mesh	.....	23	.....	+ 2.1 ± 0.36
			79.6% 325-mesh	.....	52	.....	- 1.7 ± 0.22
SYNTHETIC CALCIUM SILICATES		No. 1473 Mica.....	53.8% 325-mesh	.....	34	9.0	+ 0.8 ± 0.16
		Blue talc.....	99% 325-mesh	.....	44	8.4	- 0.9 ± 0.12
		No. 2952 Clatal.....	99.8% 325-mesh	.....	45	7.2	- 1.0 ± 0.14
		Emtal 23A.....	97.7% 325-mesh	.....	36	8.5	+ 1.4 ± 0.24
VOLCANIC MINERALS.....J-ranite K	Pyrophyllites.....	Amcolite No. 1.....	96.8% 325-mesh	.....	46	7	- 2.6 ± 0.27
		Insecticide Grade Pyrophyllite.....	93.5% 325-mesh	0.45	50	6-7	- 1.2 ± 0.22
	Montmorillonites.....	Olancha Clay.....	91% 325-mesh	130	120	6.75	- 4.8 ± 0.48
		Pikes Peak Clay.....	95.8% 325-mesh	.....	86	5.2	- 5.2 ± 0.50
		Wyoming Bentonite.....	95% 400-mesh	.....	45	8.0	- 0.5 ± 0.08
	Kaolinites.....	No. 1431 China Clay.....	99.3% 325-mesh	.....	43	4.55	- 1.5 ± 0.22
		GX Kaolin.....	99.75% 325-mesh	1.8	22	5.2	- 1.3 ± 0.20
		Barden Clay.....	Average < 1 $\mu$	23	35	5	- 1.6 ± 0.27
	Attapulgites.....	Attadlay.....	85% 325-mesh	120	119	6.9-7.1	- 5.3 ± 0.44
		Diluex A.....	90-95% 325-mesh	120-140	120	6.8-7.1	- 4.6 ± 0.53
			98.9% 325-mesh	.....	36	5.4-6.5	- 1.1 ± 0.17
	VEGETABLE ORIGIN.....	Activated carbon.....	90% 325-mesh	545	130	9-11	- 5.8 ± 0.31

\* Oil adsorption data were obtained from manufacturers, from Watkins and Norton (1955), or from tests made by the writers, but were in any case based on adsorption of linseed oil according to the rub-out test as described by Gardner (1930).

† Plastic vials with an outer surface area of 61 sq. cm. were dipped into melted beeswax, to obtain a film of wax averaging about 60  $\mu$  in thickness and about 290 mg in weight. Twenty-four hours later, the vials were mechanically shaken in 50 cc. of powder for one hour. The powder was removed by gentle washing with the aid of a cotton swab, and the quantity of wax removed by the powder was determined gravimetrically. The temperature ranged from 22° to 25° C.

‡ The addition of 0.2 per cent fatty acids has resulted in a monomolecular film of calcium soap.



TABLE 2  
EFFECT OF PRETREATMENT OF *DROSOPHILA* WITH  
DILUENTS ON THE PERIOD REQUIRED FOR 100  
PER CENT KNOCKDOWN FROM 2 PER CENT  
DIBROM IN MISSISSIPPI DILUENT\*

Diluents used in pretreatment	Period (minutes) required for 100 per cent knockdown after pretreatment in diluent for:		
	10 minutes	20 minutes	40 minutes
Walnut-shell flour.....	3	3	3
Mississippi Diluent.....	2.5	3	3
Blue talc.....	3	3.5	3
Olancha Clay.....	11	14	14
Pikes Peak Clay.....	8	17	14
Santocel C.....	9	11	11
None.....	2.5	2.5	2.5

\* Temperature, 18° C.; relative humidity, 57 per cent.

TABLE 3  
EFFECT OF PRETREATMENT OF COCKROACHES WITH  
DILUENTS ON THE PERIOD REQUIRED FOR 100 PER  
CENT KNOCKDOWN FROM 2 PER CENT DIBROM  
IN INSECTICIDE GRADE PYROPHYLLITE\*

Species of cockroach	Diluents used in pretreatment	Period (minutes) required for 100 per cent knockdown after pretreatment in diluent for:	
		10 minutes	60 minutes
German.....	Walnut-shell flour.....	22	33 <sup>b</sup>
	Pyrophyllite.....	19	17
	Pikes Peak Clay.....	8	14
	Attaclay.....	7	13
	None.....	19	..
Brown-banded.....	Walnut-shell flour.....	18	23
	Pyrophyllite.....	13	11
	Pikes Peak Clay.....	7	9
	Attaclay.....	5	8
	None.....	18	..

\* Temperature, 24° C.; relative humidity, 63 per cent.

A similar test was made with drywood termite nymphs. Only three powders were used in pretreatment, and the termites were then placed on a dust of 2 per cent Dibrom in Mississippi Diluent (treated). The average number of minutes required for 100 per cent knockdown after a ten-minute pretreatment with the various powders was as follows: SG-68, twenty-four; Insecticide Grade Pyrophyllite, thirty-two; and Mississippi Diluent, seventy-nine. Results were not significantly different when the termites were pretreated for one hour. The period required for 100 per cent knockdown when the termites were not pretreated was twenty-three minutes, approximately the same as with the sorptive powder SG-68.

### Effect of Sorptive Powders When Used as Diluents

A stock dust of 20 per cent by weight of Dibrom in Pikes Peak Clay (highly sorptive) was diluted to 2 per cent by weight of Dibrom with thirty-one of the powders listed in table 1. Since the diluents varied widely in their bulk density, the quantity of Dibrom required varied accordingly. In table 4, the quantity of toxicant required per cubic centimeter of powder is shown.

By referring back to table 1, the change in weight of films of beeswax, when shaken with the various powders in a mechanical shaker for one hour, can be found for the powders listed in table 4. The plus quantities indicate an increase in weights of the films owing to the penetration of the hard, heavy, nonsorptive particles into the wax, from which many could not be removed by washing. These particles were, on the average, the least effective in killing *Drosophila* by desiccation, with no Dibrom added, and were, on the average, the most effective diluents for Dibrom, based on the period required for 100 per cent knockdown. For the thirty-one powders tested, their effectiveness as desiccants (without Dibrom) increased, and their effectiveness as diluents for Dibrom decreased, with increasing wax sorptiveness.

In general, the same relationship was noted with respect to linseed-oil sorptiveness, although there were two notable exceptions. These were the diatomites Celite 209 and Dicalite 1G-5, which have high linseed-oil sorptiveness despite their low beeswax sorptiveness.

It is conceivable that the wax sorptiveness of the powders may have a relationship to their effect on the performance of the toxicant when they are applied to the insects *before* the toxicant is applied (tables 2 and 3). However, with reference to table 4, the apparent inverse relationship of insecticidal effectiveness to the wax sorptiveness of the powders probably is owing to the fact that the powders that adsorb wax most effectively are also those that adsorb the toxicant most effectively. Consequently, the toxicant is not readily adsorbed by the insect's cuticle, resulting in decreased effectiveness.

As stated previously, the great differences in bulk densities of the powders resulted in corresponding differences in the quantity of Dibrom per cubic centimeter of powder required for a 2 per cent concentration. Therefore, in a separate test, for every powder heavier than Pikes Peak Clay, only the quantity of Dibrom per cc. required for a 2 per cent concentration in Pikes Peak Clay was used, namely, 6.1 mg. Thus, all these diluents had the same quantity of toxicant per unit volume. This detracted very little from the ratio of superiority of the nonsorptive diluents. As can be seen in table 4, the five most sorptive diluents still required 9.7 times longer to effect a 100 per cent knockdown of *Drosophila* than the five least sorptive diluents, even though they all contained the same amount of toxicant per cc.

Table 4 also contains data pertaining to tests with the German cockroach. Again the ability of the powders to kill the insects by desiccation was generally roughly proportional to their ability to adsorb beeswax, and their effectiveness as diluents was inversely proportional to their sorptiveness. Among the highly sorptive powders, the two attapulgitites (Attaclay and Diluex A) were particularly ineffective as diluents. With 2 per cent by weight of Dibrom, they required about twenty-four times longer to effect a

TABLE 4

EFFECT OF THIRTY-ONE DILUENTS ON THE PERFORMANCE OF DIBROM AGAINST *DROSOPHILA*, AT 2 PER CENT BY WEIGHT AND AT A CONSTANT QUANTITY PER VOLUME OF DUST; AND AGAINST GERMAN COCKROACHES AT 2 PER CENT BY WEIGHT\*

Diluent†	Toxicant per cc. at 2 w./% (mg.)	<i>DROSOPHILA</i> Period (minutes) for 100 per cent knockdown with Dibrom at:			GERMAN COCKROACH Period (minutes) for 100 per cent knockdown with Dibrom at 2 w./%
		None	2 w./%	6.1 mg./cc.	
Walnut-shell flour.....	12.0	265	3	4	32
Electric Sulfur.....	16.0	134	6	8	11
Celite 209.....	3.7	160	12	..	15
Dicalite 1G-5.....	4.7	220	16	..	23
Silica 400-mesh.....	12.4	110	12	20	35
Silica DCR.....	13.4	270	4	8	17
Hi-Sil 233.....	4.7	85	13	..	31
Santocel C.....	1.5	96	17	..	64
SG-67.....	1.8	52	10	..	11
SG-68.....	2.1	90	46	..	56
Silikil D.....	12.2	120	18	22	13
Red iron oxide.....	32.4	220	5	30	42
Mississippi Diluent.....	18.4	540	4	4	25
Mississippi Diluent (treated)...	20.7	1,560	3	4	27
No. 1887 Cal. Dust A.....	25.3	185	4	6	32
Barytes.....	31.4	220	4	7	17
Blue Diamond Gypsum.....	21.0	200	4	5	15
Calcined gypsum.....	15.6	150	7	11	30
No. 1473 Mica.....	13.4	220	4	7	15
Blue talc.....	12.3	190	4	5	23
No. 2952 Clatal.....	9.5	150	7	11	28
Amcolite No. 1.....	15.4	103	14	34	143
Insecticide Grade Pyrophyllite	18.7	300	5	6	14
Olancha Clay.....	8.0	72	48	48	57
Pikes Peak Clay.....	6.1	80	42	42	72
Wyoming Bentonite.....	20.4	270	14	20	64
No. 1431 China Clay.....	10.4	137	18	22	48
GX Kaolin.....	14.2	147	7	13	30
Attaclay.....	7.8	85	54	58	343
Dilux A.....	7.8	78	55	60	363
Frianite K.....	16.4	235	6	8	20

\* For the experiment with *Drosophila*, temperature 26° C.; relative humidity, 60 per cent. For the experiment with the cockroaches, temperature, 26° C.; relative humidity, 54 per cent. In both experiments, these data refer to conditions at the beginning of the experiment.

† See table 1 for classes of the various diluents.

100 per cent knockdown of cockroaches than such nonsorptive powders as certain oxides of silicon, gypsums, sulfur, mica, and pyrophyllite.

The fluoridated silica aerogel SG-67 was the one exception to the general rule that highly sorptive powders were ineffective diluents for Dibrom when used against *Drosophila* or cockroaches. This was particularly true with respect to the latter insects, for they are especially susceptible to the contact effect of fluorides. On the other hand, No. 1431 China Clay, Amcolite No. 1, and Wyoming Bentonite proved to have an adverse effect on Dibrom, despite their relatively slight ability to adsorb wax. Therefore, one may conclude that these diluents had great ability to adsorb Dibrom, despite their low wax sorptiveness. The practical experience of the insecticide industry confirms that Wyoming Bentonite, at least, has great ability to adsorb organic toxicants.



The powders listed in table 4 were divided into five groups, with respect to wax sorptiveness, as shown in table 5. The ability of these powders to knock down both *Drosophila* and the German cockroach by desiccation, and their effectiveness as diluents for Dibrom, are shown in this table. Their ability to knock down the insects by desiccation increased, and their value as diluents decreased, with increasing sorptiveness.

In some ways, the most interesting data were obtained from the experiments with the nymphs of two species of termites (table 6). Termites die very slowly from the effects of even the most potent of available toxicants. Therefore, it is understandable that they should live as long, on the average,

TABLE 5

RELATION OF THE ABILITY OF POWDERS TO ADSORB BEESWAX, AND TO KNOCK DOWN INSECTS BY DESICCATION, TO THEIR EFFECTIVENESS AS DILUENTS FOR DIBROM\*

Group	Number of powders	Quantity of beeswax adsorbed from 60 sq. cm. of wax film (mg.)	Period for 100 per cent knockdown of <i>Drosophila</i>		Period for 100 per cent knockdown of German cockroach	
			Diluents alone (minutes)	Diluents with 2 per cent Dibrom (minutes)	Diluents alone (hours)	Diluents with 2 per cent Dibrom (minutes)
1	7	None.....	462	4.0	57.3	20.3
2	7	0.3 to 1.0.....	207	8.7	35.0	28.9
3	9	1.1 to 2.6.....	169	10.2	26.6	45.5
4	3	3.0 to 3.7.....	90	13.3	14.2	47.5
5	5	4.2 to 5.3.....	81	49.0	5.7	178.2

\* The data were obtained from table 4. The data for Silikil D and SG-67 are not included, because these diluents contain a fluoride that increases their toxicity.

when treated with sorptive powders alone as when treated with the same powders with 2 per cent by weight of Dibrom. In either case, death occurred by desiccation, and no symptoms of toxication were observable. The termites evidently could adsorb very little toxicant from the highly sorptive diluents.

Both species of termites died more rapidly in sorptive powders without toxicants than in the nonsorptive powders with toxicants. This merely shows that these insects die more rapidly from desiccation than from Dibrom toxication.

Although much time is required to kill termites with toxicants, they may become paralyzed with comparative rapidity. When treated with Dibrom in nonsorptive diluents, which generally did not interfere with adsorption of the toxicant by the insect cuticle, the tendency was for the termites to become completely motionless, appearing at first as if they had died from the effect of the toxicant. If examination was periodically continued, however, it was found that the insects eventually resumed very feeble motions of the appendages. They remained in this moribund state for a greater period than that required for death by desiccation of the termites in sorptive powders. The period required for the beginning of paralysis, i.e., the initial complete cessation of activity, averaged 0.75 hour for drywood termites and 0.36 hour for dampwood termites (table 6).

TABLE 6

EFFECT OF DILUENTS ON THE EFFECTIVENESS OF 2 PER CENT  
DIBROM DUSTS AGAINST THE NYMPHS OF DRYWOOD AND  
DAMPWOOD TERMITES\*

Diluents	Diluent alone		Diluent with 2 per cent Dibrom			
	Period for 100 per cent kill (hours)		Period to beginning of paralysis (hours)		Period for 100 per cent kill (hours)	
	Drywood termite	Damp-wood termite	Drywood termite	Damp-wood termite	Drywood termite	Damp-wood termite
(Nonsorptive)						
Walnut-shell flour.....	100.5	32.0	0.58	0.31	7.4	8.7
Silica DCR.....	12.0	12.0	1.00	0.57	6.0	9.2
Mississippi Diluent.....	85.3	26.0	0.96	0.50	8.6	8.7
Barytes.....	12.0	11.0	0.57	0.25	6.9	7.4
Blue Diamond Gypsum.....	158.7	31.0	0.50	0.23	5.4	7.6
Blue talc.....	38.8	10.1	0.88	0.33	6.4	9.2
No. 1473 Mica.....	8.2	10.7	0.61	0.30	5.0	7.5
Silikil D.....	8.0	7.8	0.90	0.43	4.9	8.3
Average.....	52.9	17.6	0.75	0.36	6.3	8.3
(Sorptive)						
SG-67.....	2.1	4.6	....†	....	2.1	4.7
Hi-Sil 233.....	3.5	5.9	....	....	2.7	6.0
Olancha Clay.....	3.6	5.3	....	....	3.1	5.3
Pikes Peak Clay.....	4.0	5.3	....	....	3.6	5.7
Attaclay.....	4.2	6.0	....	....	4.0	5.4
Diluex A.....	4.1	5.9	....	....	4.0	6.1
Average.....	3.6	5.5			3.2	5.5

\* The temperatures ranged from 20° to 29° C., and the relative humidities ranged from 60 per cent to 100 per cent. At the beginning of the experiment, the temperature was 23° C., and the relative humidity was 75 per cent.

† Paralysis never became apparent. Body movements gradually declined in intensity until time of death.

### Elutriation of the Toxicant

In the previous experiments, the dilute dusts were made by mixing inert powders with a concentrate dust of Pikes Peak Clay containing 20 per cent by weight of Dibrom. The dilutions were made with a wide variety of sorptive and nonsorptive powders. In the following experiment, an attempt was made to determine the residual toxicity of nonsorptive diluents after the Dibrom concentrate dust had been removed by air elutriation. For this purpose, unusually heavy and distinctly colored powders had to be used. A carborundum passing through a 100-mesh screen and an iron oxide dust with 90.5 per cent of its particles less than 5  $\mu$  in diameter (a theoretical 2,500-mesh) were chosen for this purpose. Elutriation was accomplished by blowing compressed air into the diluted dust formulation in a four-ounce jar. This was done in a fume hood in which the light Pikes Peak Clay-Dibrom concentrate was continuously drawn away after arising from the jar. Complete elutriation was assumed when the material left in the jar could not be distinguished from the diluent (carborundum or iron oxide), either by appearance or by odor.

Table 7 shows the ineffectiveness of carborundum and iron oxide as dust desiccants, their outstanding effectiveness as diluents for 2 per cent Dibrom (equaling that of gypsum), and their surprising degree of effectiveness even after practically all the original Pikes Peak Clay-Dibrom concentrate had been removed. This is in sharp contrast to the results with activated carbon. This is generally the most effective of all dust desiccants with which the writers have experimented, but it adsorbs Dibrom so firmly that little is available to the insect. Thus, with 2 per cent by weight of Dibrom, activated carbon was less effective than carborundum and iron oxide from which practically all the Dibrom had been removed. The extremely small quantity of Dibrom adhering to the hard, nonporous surfaces of the particles of carborundum and iron oxide was adsorbed by the insect cuticle in larger quantities than was the large amount of Dibrom held within the porous particles of the activated carbon.

TABLE 7  
TOXICITY TO *DROSOPHILA* OF DILUENTS AIR-  
ELUTRIATED FROM DUSTS CONTAINING 2 PER  
CENT DIBROM DILUTED FROM A 20 PER CENT  
MIXTURE IN PIKES PEAK CLAY\*

Materials	Period for 100 per cent knockdown (minutes)
Carborundum (100-mesh).....	360
Carborundum with 2 per cent Dibrom.....	2
Carborundum with the Dibrom elutriated.....	9
Iron oxide.....	153
Iron oxide with 2 per cent Dibrom.....	3
Iron oxide with the Dibrom elutriated.....	8
Gypsum.....	297
Gypsum with 2 per cent Dibrom.....	3
Activated carbon.....	58
Activated carbon with 2 per cent Dibrom.....	32

\* Temperature at beginning of experiment, 25° C.; relative humidity, 54 per cent.

### Difference in Rate of Decrease in Insecticidal Effectiveness of Various Dust Formulations of Dibrom When Progressively Diluted

Various concentrations of Dibrom were prepared by diluting a concentrate of 20 per cent Dibrom in Pikes Peak Clay with increasing proportions of a number of sorptive and nonsorptive powders. Their effectiveness against *Drosophila* was determined. The results are shown in table 8. The sorptive powders (SG-68, Pikes Peak Clay, and Attaclay) were less effective diluents, even with as high as 10 per cent concentrations of Dibrom. In addition, they decreased in effectiveness with subsequent dilutions at a much greater rate than the nonsorptive powders. The increases in period required for knock-down of *Drosophila* between 10 per cent concentrations and 0.2 per cent concentrations of Dibrom averaged 18.4-fold for the sorptive powders, compared



with 1.2-fold for the least sorptive of the powders (walnut-shell flour, Electric Sulfur, and Barytes). Blue talc and Barden Clay showed the effects of their moderate sorptiveness, when compared with the other "nonsorptive" powders, particularly after the dilution from 2.0 to 0.2 per cent. Barden Clay, particularly, may be considered as an intermediate between the powders of least sorptiveness and the highly sorptive powders such as the silica aerogels and certain montmorillonite and attapulgite clays.

### Comparative Tests with Various Toxicants

To determine whether the influence of the diluents might vary with different toxicants, some experiments were made in which organophosphorous toxicants (Dibrom, DDVP, parathion, malathion, and Dylox), chlorinated

TABLE 8

EFFECT OF PROGRESSIVELY GREATER DILUTIONS OF DIBROM IN SORPTIVE POWDERS, COMPARED WITH NONSORPTIVE POWDERS, WHEN USED IN DUST FORMULATIONS AGAINST *DROSOPHILA*\*

Class	Powder	Period (minutes) for 100 per cent knockdown with toxicant at:				
		10%	5%	2%	0.2%	0.0%
Botanical.....	Walnut-shell flour.....	2	2	2	2.5	285
Elemental mineral.....	Electric Sulfur.....	2	2	2	2.3	310
Gypsum.....	Barytes.....	2	2	2	2.5	344
Talc.....	Blue talc.....	2	2	3	9	283
Kaolinite.....	Barden Clay.....	3	5	7	52	336
Silica aerogel.....	SG-68.....	8	14	20	120	142
Montmorillonite.....	Pikes Peak Clay.....	5	12	21	96	110
Attapulgite.....	Attaclay.....	6	13	42	135	127

\* Temperature, 27° C.; relative humidity, 58 per cent.

hydrocarbons (chlordan, dieldrin, and lindane), and a carbamate (Sevin) were used. The experiments followed the same pattern as the preceding ones. With each insecticide, the effect of pretreatment with the sorptive and nonsorptive powders on the subsequent action of the toxic dust was determined. As before, the toxic dust was prepared with nonsorptive diluents: Mississippi Diluent (treated) or Insecticide Grade Pyrophyllite. This test was always followed by a test of the influence of the sorptive and nonsorptive powders when used as diluents for the above toxicants. The sorptive powders were Pikes Peak Clay, Attaclay, and SG-68. The nonsorptive powders were Electric Sulfur, Mississippi Diluent (treated), Barytes, Emtal 23A, and Insecticide Grade Pyrophyllite. The data in tables 9 and 10 pertain to the averages of all sorptive and all nonsorptive powders.

The results of the pretreatment experiments are shown in table 9. Contact of *Drosophila* with sorptive powders for periods of ten to forty minutes decreased their susceptibility to the organophosphorous toxicants. On the other hand, pretreatment with sorptive powders increased the susceptibility of the flies to lindane and the carbamate Sevin.

Contact of German cockroaches with the sorptive powders for periods of

ten to sixty minutes increased the susceptibility of the insects to all the toxicants used in these tests. It is apparent that the independent effect of sorptive powders may be either detrimental or beneficial, depending on the insect to be treated and the toxicant involved.

The tendency was for insects that were not pretreated in a powder to die, when exposed to the toxicant, in approximately the same period as when they were pretreated in a nonsorptive powder.

Table 10 shows that, regardless of the independent effect of the sorptive powders, as indicated in the pretreatment experiments (see table 9), when they were used as diluents, they decreased the susceptibility of *Drosophila* to all toxicants except Sevin and lindane.

In the experiments with the German cockroach, the data for dieldrin and chlordane are not applicable to the present problem because the slowness of their toxic action resulted in death by desiccation, when sorptive diluents were used, long before toxication became evident. The period required to knock down and kill cockroaches with chlordane or dieldrin in sorptive diluents was never significantly different from the period required to knock them down or kill them with the corresponding sorptive powders alone. However, when no toxicant is present, a certain percentage of the cockroaches have a tendency to die without first lying on their backs. Therefore, when they no longer move about, they must be forcibly turned so as to lie on their backs. If they can no longer get back on their feet, they are considered to be "knocked out."

The sorptive powders, when used as diluents, decreased the susceptibility of the cockroaches to the organophosphorous toxicants, had no effect with respect to lindane, and increased their susceptibility to Sevin.

It is noteworthy that regardless of their independent action in pretreatment, the sorptive powders always decreased the effectiveness of the organophosphorous toxicants with both species of test insects. This adverse effect of the sorptive powders, when used as diluents, is believed to be the result of their ability to adsorb the toxicant so firmly that it is not readily available to the insect. It may logically be assumed that the toxicants that are liquids in their pure state would be the most adversely affected by adsorption. Among the toxicants used, Dibrom, DDVP, parathion, malathion, Dylox, and chlordane are liquids, although chlordane is rather viscous. Dylox is a solid in the pure state, but remained in a nonvolatile solvent as used in the present experiments.

Lindane and Sevin, which are crystalline solids in the pure state, were not adversely affected by sorptive diluents and, in fact, Sevin was benefited in the test with cockroaches (table 9). The crystalline solids are possibly subject to a certain amount of adsorption, but the independent action of the sorptive diluent may be beneficial and balance the adverse effect of adsorption. This would be most apt to be the case with Sevin and lindane, for their action against both *Drosophila* and cockroaches was markedly enhanced by pretreatment of the insects with sorptive powders.

Of all the toxicants used, Dibrom, DDVP, and Dylox were by far the most adversely affected by sorptive diluents. With Dibrom, the ratio of superiority of the nonsorptive diluents, when compared with the sorptive diluents, was

TABLE 9

EFFECTS OF PRETREATMENT OF INSECTS WITH SORPTIVE AND NONSORPTIVE POWDERS ON THE EFFECTIVENESS  
OF VARIOUS TOXICANTS AGAINST *DROSOPHILA* AND COCKROACHES\*

Insect	Toxicant	Concentration (per cent)	Period of pre- treatment (minutes)	Temperature (°C.)	Relative humidity (per cent)	Period (minutes) for 100 per cent knockdown when pretreated with:		Difference	L.S.D. at $p = 0.05$
						Sorptive powder†	Nonsorptive powder‡		
<i>Drosophila</i> .....	Dibrom.....	0.2	10	24	57	9	3	6	1
	Dibrom.....	0.2	20	24	57	14	3	11	3
	Dibrom.....	0.2	40	24	57	13	3	10	1
	DDVP.....	1	20	25	50	11	1	10	2
	Parathion.....	2	25	24	27	33	23	10	4
	Malathion.....	2	25	24	49	33	14	19	4
	Dylox.....	5	25	21	36	36	15	21	5
	Lindane.....	2	20	25	50	9	14	5	2
	Sevin.....	2	25	27	22	21	37	16	5
	Dibrom.....	2	10	24	63	7	21	14	2
German cockroach.....	Dibrom.....	2	60	24	63	13	25	12	7
	DDVP.....	1	20	22	12	7	10	3	2
	Parathion.....	2	10	24	43	36	42	6	6
	Malathion.....	2	10	25	38	45	65	20	11
	Dylox.....	5	20	22	40	64	91	27	17
	Lindane.....	2	15	24	38	15	26	11	8
	Sevin.....	2	10	24	32	14	42	28	8
	Dibrom.....	2	10	24	63	7	21	14	2
	Dibrom.....	2	60	24	63	13	25	12	7
	DDVP.....	1	20	22	12	7	10	3	2

\* The insects were placed in 1 cc. of diluent in four-ounce jelly jars, then screened and blown free of excess diluent before being placed in a mixture of toxicant and nonsorptive diluent.

† The sorptive powders were Pikes Peak Clay, Attaclay, and the silica aerogel SG-48.

‡ The nonsorptive powders were Electric Sulfur, Mississippi Diluent (treated), Barytes, Emtal 23A, and Insecticide Grade Pyrophyllite.



TABLE 10  
COMPARATIVE PERFORMANCES OF VARIOUS DILUENTS WITH DIFFERENT TOXICANTS AGAINST  
*DROSOPHILA* AND COCKROACHES\*

Insect	Toxicant	Concen- tration (per cent)	Temperature (°C.)	Relative humidity (per cent)	Period (minutes) for 100 per cent knockdown when toxicant diluted with:		Difference	L.S.D. at $p = 0.05$
					Sorptive diluent	Nonsorptive diluent		
<i>Drosophila</i> .....	Dibrom.....	2	26	60	47	5	42	4
	DDVP.....	1	24	29	52	1	51	11
	Parathion.....	2	24	36	47	19	28	6
	Malathion.....	2	26	21	54	12	42	6
	Dylox.....	5	22	40	62	7	55	13
	Chlordane.....	5	25	54	71	63	8	7
	Dieldrin.....	5	23	44	100	76	24	18
	Lindane.....	2	22	44	30	32	2	5
	Sevin.....	2	24	54	37	34	3	7
	Dibrom.....	2	26	52	157	18	139	46
	DDVP.....	1	22	41	93	7	86	34
	Parathion.....	2	26	18	48	36	12	3
German cockroach .....	Malathion.....	2	25	19	60	36	24	18
	Dylox.....	5	22	21	242†	93	..	..
	Chlordane.....	5	22	50	203†	1,356	..	..
	Dieldrin.....	5	23	48	330†	1,485	..	..
	Lindane.....	2	22	48	34	33	1	6
	Sevin.....	2	22	50	18	40	22	7

\* The insects were placed in 1 cc. of toxic powder in four-ounce jelly jars. The sorptive and nonsorptive diluents were the same as those used in the experiments shown in table 9.

† The cockroaches appeared to have died of desiccation, so the effect of the diluent on the toxicant could not be determined.

9.4 to 1 with *Drosophila* and 8.2 to 1 with the German cockroach. With DDVP, the superiority of the nonsorptive diluents was 52 to 1 with *Drosophila* and 13.3 to 1 with the German cockroach. With Dylox, the superiority of the nonsorptive diluents was 8.9 to 1 with *Drosophila* and over 2.6 to 1 with the cockroach. Death of the cockroaches occurred before toxication took place.

TABLE 11  
COMPARATIVE PERFORMANCE OF A PYROPHYLLITE AND A  
MONTMORILLONITE CLAY WITH EQUAL QUANTITIES  
OF TOXICANT PER VOLUME OF DILUENT\*

Insect	Toxicant	Pikes Peak Clay formulations		Insecticide Grade Pyrophyllite formulations		Difference (minutes)	L.S.D. at $p = 0.05$
		Per cent by weight	100 per cent knockdown (minutes)	Per cent by weight	100 per cent knockdown (minutes)		
<i>Drosophila</i> ....	Dibrom.....	4	22	2	5	17	3
	DDVP.....	2	16	1	3	13	2
	Parathion.....	4	39	2	18	21	4
	Malathion.....	4	36	2	16	20	4
	Dylox.....	10	63	5	10	53	6
	Lindane.....	4	14	2	14	0	3
	Chlordane.....	10	55	5	58	3	8
	Dieldrin.....	10	56	5	54	2	8
German cockroach..	Sevin.....	4	40	2	38	2	6
	Dibrom.....	4	18	2	12	6	3
	DDVP.....	2	14	1	8	6	3
	Parathion.....	4	35	2	37	2	5
	Malathion.....	4	28	2	30	2	4
	Dylox.....	10	66	5	58	8	8
	Lindane.....	4	28	2	30	2	5
	Chlordane.....	10	117†	5	436	..	..
	Dieldrin.....	10	120†	5	450	..	..
	Sevin.....	4	9	2	12	3	2

\* Pikes Peak Clay has approximately one-half the bulk density of Insecticide Grade Pyrophyllite; therefore, the per cent by weight of toxicant was made twice as high in the Pikes Peak Clay formulation in order to insure the same quantity of toxicant per unit volume in the two formulations. Temperature, 23° C.; relative humidity, 45 per cent.

† The cockroaches were knocked down and killed by desiccation before any appreciable toxic action could take place. The period for 100 per cent knockdown for cockroaches treated with Pikes Peak Clay alone was 118 minutes and, for pyrophyllite alone, twenty-one hours.

### Diluents with Equal Quantities of Toxicant per Unit Volume

It is shown in table 4 that when an equal quantity of Dibrom was used per unit volume of sorptive and nonsorptive powders, the latter were still greatly superior as diluents. An experiment was made to determine if this would hold true for certain other toxicants.

It happens that the bulk density of Insecticide Grade Pyrophyllite (nonsorptive) is practically twice that of Pikes Peak Clay (sorptive). Therefore, if toxicants are used in twice the concentration in the latter diluent, the quantity per unit volume will be approximately the same in the two diluents. Nine toxicants were added to Pikes Peak Clay in two times higher concentration than to Insecticide Grade Pyrophyllite. Table 11 shows that, when used against *Drosophila*, the pyrophyllite was superior to clay when organophos-

phorous toxicants were added, but was no better than clay as a diluent for the other toxicants. With the German cockroach as the test insect, pyrophyllite was superior as a diluent for Dibrom, DDVP, and Dylox, but for no other toxicants. On the other hand, the clay proved to be more effective than the pyrophyllite as a diluent for Sevin.

In both tables 10 and 11, Sevin was more effective in sorptive than in nonsorptive diluents when used against cockroaches, whereas no significant differences were found between the two types of diluent when Sevin was used against *Drosophila*. This may result from the beneficial independent effect of the sorptive diluents, and adverse effect with the nonsorptive diluents, when the cockroach is used as the test insect (see table 9). However, in one experiment made independently of those depicted in tables 10 and 11, Sevin proved to be more effective in Pikes Peak Clay than in Insecticide Grade Pyrophyllite, both against *Drosophila* and against the German cockroach. At 2 per cent concentration in the clay, Sevin knocked down *Drosophila* in forty-six minutes; at 4 per cent in clay, forty minutes; and at 2 per cent in pyrophyllite, sixty-six minutes. The corresponding figures when the German cockroach was used as the test insect were eleven, ten, and eighteen, respectively. The temperature was 27° C., and the relative humidity was 32 per cent.

### Comparison of Sorptive and Nonsorptive Diluents in Old Residues

An experiment was made to determine whether the superiority of the sorptive powders as diluents for organophosphorous toxicants continues with increasing age of the residues of the toxic dusts. Formulations of 1 per cent DDVP in Attaclay and in Insecticide Grade Pyrophyllite were prepared. *Drosophila* and German cockroaches were placed in 1 cc. of each of these formulations in four-ounce jelly jars, as in previous experiments. After each test, the insects were removed but the powders remained. Since there were only four sets of jars for each insect species, and the tests were made at weekly intervals, each jar had to be used twelve times during the experiment, which was begun on December 26, 1959, and completed on March 22, 1960. The experiment was made in a well-lighted room with a mean temperature of 23° C. and a mean relative humidity of 39 per cent.

The increase in period required for 100 per cent knockdown with increasing age of the toxic residues is shown in table 12.

DDVP showed a remarkable degree of persistence in pyrophyllite, in view of the brief period of residual activity generally displayed by this insecticide under ordinary conditions of usage. It was not possible to compare the performance of this toxicant in the two types of diluent over the entire three-month period, because with *Drosophila*, death by desiccation took place in the sorptive diluent before toxic action was apparent, even on the first day of the experiment. With cockroaches, the toxic action of the DDVP had decreased during the third month of the experiment to such an extent that again the insects were killed by desiccation before any toxic action was evident.



TABLE 12

INCREASE IN PERIOD REQUIRED FOR 100 PER CENT  
KNOCKDOWN OF *DROSOPHILA* AND MALE GERMAN  
COCKROACHES IN RESIDUES OF 1 PER CENT DDVP  
IN A SORPTIVE AND IN A NONSORPTIVE DILUENT  
OVER A PERIOD OF THREE MONTHS\*

Insect	Diluent	Average period (minutes) for 100 per cent knockdown during:		
		First month	Second month	Third month
<i>Drosophila</i> .....	Attaclay.....	83†	79†	88†
	Pyrophyllite.....	3.8	5.2	7.8
German cockroach..	Attaclay.....	34	92	187†
	Pyrophyllite.....	6.2	20.5	32.3

\* The experiments lasted from December 26, 1959, to March 22, 1960. The mean temperature for that period was 23° C., and the mean relative humidity was 39 per cent.

† The insects died from desiccation, resulting from the adsorption of the lipid from their epicuticles by the diluents, before death by toxication could take place.

## DISCUSSION

The choice of the diluents in the preparation of insecticide dusts has traditionally been influenced by the following factors: (1) chemical compatibility with the toxicant, (2) bulk density, (3) particle size and distribution, (4) flowability, (5) dustability, (6) abrasiveness, (7) adsorptivity, and (8) price and availability.

Adsorptivity has been considered in relation to the amount of toxicant that can be incorporated into the diluent. In the case of liquid toxicants, it is essential that the desired concentration of toxicant be adsorbed without impairing the flowability and dustability of the final product. In the present paper, it is shown that adsorptivity of the diluent is of importance not only in relation to formulation problems, but also in relation to its adverse effect on the insecticidal efficiency of dusts containing liquid toxicants. This effect becomes increasingly important with decreasing concentration of the toxicant in the dilute dust.

It is usually necessary to use highly sorptive diluents for the preparation of dust concentrates, for the quantity of toxicant in these concentrates may vary from 20–25 per cent up to 75 per cent by weight. However, it would appear that nonsorptive diluents could be used to advantage in diluting concentrates with liquid toxicant down to the percentage used in field application, because of the greater insecticidal efficiency of formulations containing such diluents. This presumes, of course, that the nonsorptive diluents would have the other desirable characteristics listed above, besides their nonsorptivity.

In view of the undesirable characteristic of sorptive diluents of preventing free access of the liquid toxicants to the insect cuticle, it would appear to be desirable whenever possible to spray such toxicants directly onto a nonsorptive diluent in the quantity used in the ultimate dust formulation. This would appear to be particularly desirable with toxicants on which sorptive

diluents have an especially adverse effect, such as Dibrom, DDVP, and Dylox. On the other hand, it appears that most toxicants that are solid in the pure or technical state are affected little, if any, by the degree of sorptiveness of the diluent; the effectiveness of Sevin against cockroaches is actually *increased* by the use of a highly sorptive diluent.

The writers are aware that the pH of the diluent may have some influence on the insecticidal effectiveness of a toxicant. The pH's of the various powders used in this investigation are given in table 1. This table shows that there was a wide range of pH among both sorptive and nonsorptive powders, yet this did not prevent their acting as a class, with regard to their effect on toxicants, when they were used as diluents.

## SUMMARY

When adult *Drosophila pseudoobscura* were placed on "sorptive" powders (Olancha Clay, Pikes Peak Clay, and Santocel C) before being placed in a 2 per cent Dibrom dust, the period required to bring about their "knock-down" was 3.3 times longer than when they were placed in "nonsorptive" powders—walnut-shell flour, Mississippi Diluent (treated), and blue talc—or when they were not "pretreated."

With German and brown-banded cockroaches, pretreatment with sorptive powders had an effect that was directly opposite to that obtained with *Drosophila*. A ten-minute pretreatment with the sorptive powders caused the German cockroaches to be knocked down in 60.6 per cent less time, and the brown-banded cockroaches in 66.7 per cent less time, than when they were pretreated with nonsorptive powders.

In the pretreatment experiments with drywood termites, results were similar to those obtained with cockroaches.

Regardless of the independent effect of the sorptive powders on the susceptibility of the insects to Dibrom, as indicated by the pretreatment experiments, they were much less effective than the nonsorptive powders as diluents for Dibrom when effectiveness was based on the period required for paralysis or knockdown. This was the case with all insect species tested: two species of cockroaches and termites and one species of *Drosophila*. The reason appears to be that the sorptive powders, when used as diluents, adsorb the toxicant so effectively that it cannot be adsorbed by the insect cuticle in quantities adequate for rapid toxication.

In the case of the two species of termites, when sorptive powders were used as diluents for Dibrom, toxication was retarded to such an extent that the insects died of desiccation before signs of paralysis became apparent. They died no more rapidly than did the termites treated with the same powders without the addition of Dibrom. On the other hand, when nonsorptive powders were used as diluents, paralysis occurred rapidly. However, the period required for death to occur was greater than when the sorptive powders were used as diluents, for the latter killed the insects by desiccation. Desiccation resulted in the death of termites more rapidly than toxication.

With both cockroaches and termites, the independent effect of the sorptive powders was to increase the rate of toxic action of the subsequently applied



Dibrom, yet these powders proved to be the least effective diluents because they tended to retard the release of the toxicant to the insect cuticle.

When 2 per cent Dibrom was added to powders of carborundum and iron oxide (both nonsorptive) and subsequently removed by air elutriation, the remaining powder was far more toxic to *Drosophila* than an unelutriated dust of 2 per cent Dibrom in activated carbon (highly sorptive).

When formulations of 10 per cent Dibrom in powders of various degrees of adsorptiveness were diluted progressively down to 0.2 per cent, those in the three least sorptive powders decreased only 20 per cent, whereas those in the three most sorptive powders decreased 18-fold, in rate of toxic action against *Drosophila*.

Following the experiments with Dibrom as the test toxicant, a comparative study of various toxicants was made. Using *Drosophila* as the test insect, pretreatment with sorptive powders decreased the rate of toxic action of the organophosphorous compounds, but increased the rate of toxic action of lindane and Sevin. When the German cockroach was used as the test insect, pretreatment with sorptive powders increased the rate of toxic action of all toxicants.

When used as diluents, the sorptive powders decreased the rate of toxic action of all organophosphorous compounds against both *Drosophila* and the German cockroach. However, there was no difference in the effect of sorptive and nonsorptive diluents when lindane was used as the toxicant. With Sevin, the sorptive diluents increased the rate of toxic action against the German cockroach, but were not significantly different from the nonsorptive diluents against *Drosophila*. Chlordane and dieldrin could not be tested in this respect against German cockroaches because the sorptive diluents resulted in their death by desiccation before toxication could take place. When used against *Drosophila*, they resulted in knockdown more rapidly when diluted with the nonsorptive diluents.

As might be expected, the sorptive diluents had the most deleterious effect when used with toxicants that were liquid in the pure or technical state, which included all the organophosphorous toxicants used in the experiments. However, among the organophosphorous compounds, Dibrom, DDVP, and Dylox were far more adversely affected than were parathion and malathion. The ratios of periods (minutes) required for 100 per cent knockdown with sorptive diluents compared with nonsorptive diluents were as follows: with 2 per cent Dibrom against *Drosophila*, 9.4 to 1, and against the German cockroach, 8.2 to 1; with 1 per cent DDVP against *Drosophila*, 52 to 1, and against the German cockroach, 13.3 to 1; with 5 per cent Dylox against *Drosophila*, 8.9 to 1, and against the German cockroach, over 2.6 to 1 (the sorptive diluents caused death by desiccation).

In one experiment, Dibrom was used at 2 per cent concentration by weight in Insecticide Grade Pyrophyllite and 4 per cent in Pikes Peak Clay, so as to provide equal quantities of toxicant per unit volume, for the pyrophyllite has twice the bulk density of the clay. Under these conditions, the pyrophyllite mixture was still superior as an insecticide when the organophosphorous toxicants were used.

Residues of 2 per cent dust concentrations of DDVP in Insecticide Grade



Pyrophyllite and in Pikes Peak Clay were allowed to age indoors for a period of three months. The period required for 100 per cent knockdown of *Drosophila* with the DDVP-pyrophyllite dust increased from 3.8 to 7.8 minutes during that period, while with the DDVP-clay dust, death by desiccation took place before the paralyzing effect of the toxicant was evident. The period for 100 per cent knockdown of the German cockroach with the DDVP-pyrophyllite dust increased from 6.2 to 32.3 minutes during the three months of the experiment, while with the DDVP-clay dust, it increased from 34 to 187 minutes. The latter figure was not significantly different from the period required to knock down cockroaches by means of the clay without toxicant. There was no longer sufficient toxicant present to cause a paralysis of the insect before desiccation resulting from the action of the sorptive diluent.

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## LITERATURE CITED

- ALEXANDER, P., J. A. KITCHENER, and H. V. A. BRISCOE  
1944. Inert dust insecticides. *Ann. Appl. Biol.*, **31**:143-59.
- EBELING, W., and R. E. WAGNER  
1959. Rapid desiccation of drywood termites with inert sorptive dusts and other substances. *Jour. Econ. Ent.* **52**:190-207.
- GARDNER, H. A.  
1930. Physical and chemical examination of paints, varnishes, lacquers, and colors. Fifth edition. Institute of Paint and Varnish Research, Washington, D.C.
- GLYNNE JONES, G. D.  
1955. The cuticular waterproofing mechanism of the worker honeybee. *Jour. Exptl. Biol.* **32**:95-109.
- HELVEY, T. C.  
1952. Insecticidal effect of inert solid diluents. *Science* **116**:631-32.
- HURST, H.  
1948. Asymmetrical behavior of insect cuticle in relation to water permeability. *Disc. Faraday Soc.* **3**:193-210.
- MICKS, D. W.  
1960. Susceptibility of mosquitoes to silica gel insecticides. *Jour. Econ. Ent.* **53**:915-18.
- NAIR, M. R. G. K.  
1957. Structure of waterproofing epicuticular layers in insects in relation to inert dust action. *Indian Jour. Ent.* **10**(1):37-49.
- TARSHIS, I. B.  
1959. U.C.L.A. tests with desiccant dusts for roach control. *Pest Control* **27**:16-18, 20, 22, 24, 26-28, 30, 32.  
1960. Control of the snake mite (*Ophionyssus natricis*), other mites, and certain insects with the sorptive dust, SG-67. *Jour. Econ. Ent.* **53**:903-8.  
1961. Laboratory and field evaluation studies with sorptive dusts for the control of arthropods affecting man and animal. *Exptl. Parasitol.* **11**: February-March.
- WAGNER, R. E., and W. EBELING  
1959. Lethality of inert dust materials to *Kaloterms minor* Hagen and their role as preventives in structural pest control. *Jour. Econ. Ent.* **52**:207-12.
- WATKINS, T. C., and L. B. NORTON  
1955. Handbook of insecticide dust diluents and carriers. Second edition, revised by D. E. Weidhaas and J. L. Brann, Jr. Dorland Books, Caldwell, N.J. 233 pp.